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(54) Title: THERAPEUTIC COMPOSITIONS INCLUDING PROTEIN KINASE C INHIBITORS

(57) Abstract: Compositions comprising a PKC inhibitor, or a pharmaceutically acceptable salt thereof, and an antioxidant, essential fatty acid, or a prostacyclin agent, or a pharmaceutically acceptable salt thereof are provided. Also provided are methods of treatment comprising administration of such compositions, and methods of treatment comprising co-administration of a PKC inhibitor, or a pharmaceutically acceptable salt thereof, and an antioxidant, essential fatty acid, or a prostacyclin agent, or a pharmaceutically acceptable salt thereof.

Therapeutic Compositions Including Protein Kinase C Inhibitors

Background of the Invention

Protein kinase C (PKC) consists of a family of closely related enzymes that function as serine/threonine kinases. Protein kinase C plays an important role in cell-cell signaling, gene expression, and in the control of cell differentiation and growth. At present, there are currently at least ten known isozymes of PKC that differ in their tissue distribution, enzymatic specificity, and regulation. Nishizuka Y. Annu. Rev. Biochem. 58: 31-44 (1989); Nishizuka Y. Science 258: 607-614 (1992).

Protein kinase C isozymes are single polypeptide chains ranging from 592 to 737 amino acids in length. The isozymes contain a regulatory domain and a catalytic domain connected by a linker peptide. The regulatory and catalytic domains can be further subdivided into constant and variable regions. The catalytic domain of protein kinase C is very similar to that seen in other protein kinases while the regulatory domain is unique to the PKC isozymes. The PKC isozymes demonstrate between 40-80% homology at the amino acid level among the group. However, the homology of a single isozyme between different species is generally greater than 97%.

Protein kinase C is a membrane-associated enzyme that is allosterically regulated by a number of factors, including membrane phospholipids, calcium, and certain membrane lipids such as diacylglycerols that are liberated in response to the activities of phospholipases. Bell, R.M. and Burns, D.J., J. Biol. Chem. 266: 4661-4664 (1991); Nishizuka, Y. Science 258: 607-614 (1992). The protein kinase C isozymes, alpha, beta-1, beta-2 and gamma, require

membrane phospholipid, calcium and diacylglycerol/phorbol esters for full activation. The delta, epsilon, eta, and theta forms of PKC are calcium-independent in their mode of activation. The zeta and lambda forms of PKC are independent of both calcium and diacylglycerol and are believed to require only membrane phospholipid for their activation.

Only one or two of the protein kinase C isozymes may be involved in a given disease state. For example, the elevated blood glucose levels found in diabetes lead to an isozyme-specific elevation of the beta-2 isozyme in vascular tissues. Inoguchi et al., Proc. Natl. Acad. Sci. USA 89: 11059-11065 (1992). A diabetes-linked elevation of the beta isozyme in human platelets has been correlated with their altered response to agonists. Bastyr III, E.J. and Lu, J. Diabetes 42: (Suppl. 1) 97A (1993). The human vitamin D receptor has been shown to be selectively phosphorylated by protein kinase C beta. This phosphorylation has been linked to alterations in the functioning of the receptor. Hsieh et al., Proc. Natl. Acad. Sci. USA 88: 9315-9319 (1991); Hsieh et al., J. Biol. Chem. 268: 15118-15126 (1993). In addition, recent work has shown that the beta-2 isozyme is responsible for erythroleukemia cell proliferation while the alpha isozyme is involved in megakaryocyte differentiation in these same cells. Murray et al., J. Biol. Chem. 268: 15847-15853 (1993).

The ubiquitous nature of the protein kinase C isozymes and their important roles in physiology provide incentives to produce highly selective PKC inhibitors. Given the evidence demonstrating linkage of certain isozymes to disease states, it is reasonable to assume that inhibitory compounds that are selective to one or two protein kinase C isozymes relative to the other PKC isozymes and other protein kinases are superior therapeutic agents. Such compounds should demonstrate greater efficacy and lower toxicity by virtue of their specificity.

The microbial indolocarbazole, staurosporine, is a potent inhibitor of protein kinase C that interacts with the catalytic domain of the enzyme. Tamaoki et al., Biochem. Biophys. Res. Commun. 135: 397-402 (1986); Gross et al., Biochem. Pharmacol. 40: 343-350 (1990). However, the therapeutic usefulness of this molecule and closely related compounds is limited by the lack of specificity for protein kinase C over other protein kinases. Ruegg, U.T. and Burgess, G.M., Trends Pharmacol. Sci. 10: 218-220 (1989). This lack of selectivity results in unacceptable toxicity in this class of molecules.

An additional class of compounds related to staurosporine, the bisindolemaleimides, has been the focus of recent work. Davis et al., FEBS Lett. 259: 61-63 (1989); Twoemy et al., Biochem. Biophys. Res. Commun. 171: 1087-1092 (1990); Toullec et al., J. Biol. Chem. 266: 15771-15781 (1991); Davis et al., J. Med. Chem. 35: 994-1001 (1992); Bit et al., J. Med. Chem. 36: 21-29 (1993). Some of these compounds have demonstrated selectivity for protein kinase C over other protein kinases.

Although compounds that demonstrate specificity to protein kinase C have been discovered, very little is known regarding isozyme selectivity. For example, analysis of the isozyme selectivity of staurosporine, shows little isozyme selectivity with the exception of poor inhibition of the zeta isozyme relative to the other isozymes. McGlynn et al., J. Cell. Biochem. 49: 239-250 (1992); Ward, N.E., and O'Brian, C.A., Molec. Pharmacol. 41: 387-392 (1992). Studies of the PKC-selective compound, 3-[1-(3-dimethylaminopropyl)-indol-3-yl]-4-(1H-indol-3-yl)-1H-pyrrole-2,5-dione, suggest a slight selectivity for the calcium dependent isozymes. Toullec et al., J. Biol. Chem. 266: 15771-15781 (1991). Subsequent studies of this compound observed no difference, or possibly slight selectivity, for alpha over beta-1 and beta-2 isozymes. Martiny-Baron et al., J. Biol. Chem. 268: 9194-9197 (1993);

Wilkinson, et al., Biochem. J. 294: 335-337 (1993). Therefore, despite years of research and the identification of classes of compounds that inhibit protein kinase C versus other protein kinases, there remains a need for therapeutically effective isozyme-selective inhibitors.

Isozyme selective inhibitors of protein kinase C are useful in treating conditions associated with diabetes mellitus and its complications, including diabetic neuropathy and vascular complications, erectile dysfunction, ischemia, inflammation, central nervous system disorders, cardiovascular disease, Alzheimer's Disease, dermatological disease and cancer (see for example U.S. Patents Nos. 5,552,396, 5,723,456, 5,780,461, 5,624,949, 5,674,862, 5,719,175, 5,545,636, 5,668,152, 5,672,173, 5,481,003, 5,661,173, 5,672,618 and 5,491,242, all incorporated herein by reference).

Diabetes mellitus is one of the commonest causes of neuropathy, which has clinical manifestations in the majority of patients with a disease duration of greater than ten years. Peripheral sensorimotor and autonomic neuropathies are the main manifestation of damage in diabetes, but less commonly, symmetric and mononeuropathies are found. Sensory symptoms include deficits in perception of fine touch and vibration, accompanied by abnormalities of thermal discrimination and sometimes by severe pain (painful neuropathy). Sensorimotor reflexes are reduced. Autonomic symptoms affect major organ systems including the regulation of heart and blood pressure; there are gastric disturbances, changes in sweating, and male impotence. Early following diabetes diagnosis, evidence of nerve dysfunction is seen by reduced motor and sensory conduction velocity and increased resistance to ischemic conduction failure which precede the occurrence of overt nerve fiber damage.

The etiology of these changes is complex and multifactorial. Measurements on diabetic patients and animal models such as the streptozotocin-induced diabetic rat, have shown that vascular and metabolic factors are involved. Nerve blood flow and endoneurial oxygen tensions are diminished by diabetes, and this has been linked to glucose-dependent metabolic changes in the blood vessels. A number of mechanisms have been identified, including polyol pathway activation, nonenzymatic glycation of proteins and the formation of advanced glycation end products, increased oxygen free radical activity, impaired ω-6 essential fatty acid and prostanoid metabolism, reduced nerve L-carnitine levels, and abnormalities in protein kinase C (PKC) activation by diacylglycerol (DAG). The potential importance of elevated PKC activity in diabetic neuropathy, and particularly the β-isoform has been shown in diabetic rats.

We have now identified unexpected synergistic interactions between PKC inhibitors and other agents that are important therapeutically.

For example, the treatment of diabetic rats with low doses of either a PKC inhibitor, an antioxidant or the ω-6 essential fatty acid, γ-linolenic acid (GLA) had modest effects on motor and sensory conduction velocity and sciatic nerve blood flow, with 19-25% correction of diabetic deficits. However, when the same dose of a PKC inhibitor was given jointly with either of these agents, nerve conduction and blood velocity was restored to the nondiabetic range.

In terms of known mechanisms of drug action, the antioxidants vitamin E and α-lipoic acid would be expected to reduce nerve and blood vessel damage by reactive oxygen species. It is not clear how this affects the PKC system,

but reactive oxygen species under some conditions may stimulate protein kinase C, therefore vitamin E and alpha-lipoic acid, by reducing PKC activation, would be expected to have only an additive effect with a PKC inhibitor. Furthermore, vitamin E in some vascular tissues increases the activity of DAG kinase, which would remove excess DAG and therefore reduce PKC activation. Therefore, the prediction is that there would be an additive effect with the PKC inhibitor.

However, for these drug combinations, the effect on nerve function greatly exceeded that predicted from a simple addition of individual drug effects, indicating a marked synergistic interaction. Thus, rather than reproducing the effect of approximately twice the PKC inhibitor dose, the drug interactions unexpectedly produced effects on conduction velocity that would have required dose of PKC inhibitor of 20 to 60 times greater.

Gamma-linoleic acid (GLA) is not an antioxidant, but has a major action as a substrate for arachidonic acid and prostanoid production. Existing evidence shows that GLA's action against experimental diabetic neuropathy is blocked by cyclooxygenase inhibition, implicating the increased production of vasoactive prostanoids such as prostacyclin that have beneficial effects on nerve blood flow and conduction velocity. Up to now, there has been no established link between this action and PKC activity.

For both the antioxidants and GLA, a synergistic interaction with a PKC inhibitor could not have been predicted from preexisting data. Therefore, the synergy between antioxidants or GLA and a PKC inhibition provides a novel and unexpected therapeutic application for neural and neurovascular disease states where PKC is activated. These include diabetic neuropathy, peripheral vascular disease, ischemic and ischemia-reperfusion damage to nerves and

neural tissue, and other complications of diabetes where nerve and vascular dysfunction contribute to the etiology, including the problems of diabetic foot, ulceration and impaired healing.

Like vitamin E, probucol and butylated hydroxytoluene are lipophilic free radical scavengers. Naturally occuring lipophilic free radical scavengers include beta-carotene, lycopene, flavenoids (such as silybin, diosmin, hesperidin, and delphinidin) and ubiquinol (coenzyme Q). Lazaroids (such as tirilazad, and 21-aminosteroids) are also lipid soluble antioxidants.

Lipoic acid is both lipid and water soluble. Other hydrophilic free radical scavengers include vitamin C and superoxide dismutase and its synthetic analogues. Lipoic acid is reduced in the body to form dihydrolipoic acid which is probably the main scavenging form for certain importain free radicals, particularly the hydroxyl radical. This reducing power involves sulphydryl groups. Sulphydryl donors include acetyl-L-cysteine and glutathione. Dimethylthiourea is a specfic hydroxyl scavenger.

Lipoic acid chelates transition metals, which catalyze certain free radical forming reactions. Transition metal chelator antioxidants include deferoxamine and trientine. The formation of advanced glycation end products (AGEs) is accelerated by free radicals and is also a source of them in reactions catalyzed by transition metals (the so-called glycoxidation or autoxidative glycosylation process). Agents which prevent AGE formation include aminoguanidine and its derivatives.

N-6 essential fatty acids work by improving prostacyclin production. Agents which improve prostacyclin production, or potentiate prostacyclin action include type III phosphodiesterase inhibitors such as cilostazol.

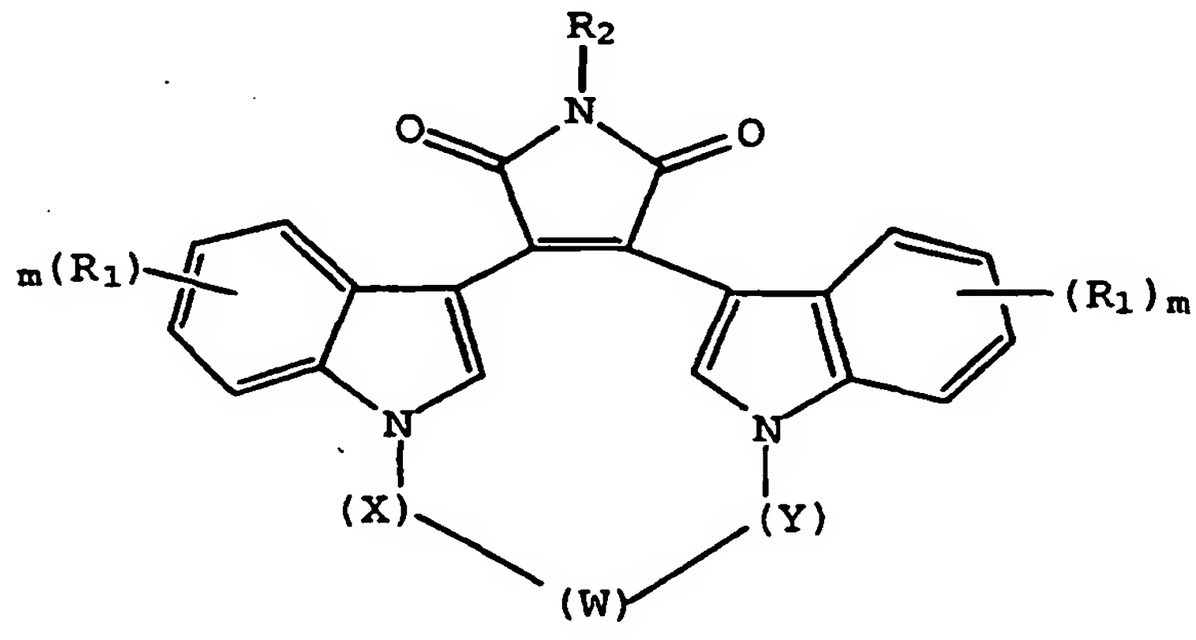
Prostaglandin analogues include iloprost, and beraprost sodium.

Summary of the Invention

The present invention provides for compositions comprising a PKC inhibitor, or a pharmaceutically acceptable salt thereof, and an antioxidant, essential fatty acid, or a prostacyclin agent, or a pharmaceutically acceptable salt thereof. The present invention also provides for methods of treatment comprising administration of such compositions, and methods of treatment comprising co-administration of a PKC inhibitor, or a pharmaceutically acceptable salt thereof, and an antioxidant, essential fatty acid, or a prostacyclin agent, or a pharmaceutically acceptable salt thereof.

Detailed Description of the Invention

A preferred embodiment of the present invention provides compositions comprising compounds of Formula I:



(I)

wherein:

W is -O-, -S-, -SO-, -SO_2-, -CO-, C_2-C_6 alkylene, substituted alkylene, C_2-C_6 alkenylene, -aryl-, -

aryl$(CH_2)_m$O-, -heterocycle-, -heterocycle-$(CH_2)_m$O-, -fused bicyclic-, -fused bicyclic-$(CH_2)_m$O-, -NR_3-, -NOR_3-, -CONH-, or -NHCO-;

X and Y are independently C_1-C_4 alkylene, substituted alkylene, or together X, Y, and W combine to form -$(CH_2)_n$-AA-;

R_1 is independently hydrogen, halo, C_1-C_4 alkyl, hydroxy, C_1-C_4 alkoxy, haloalkyl, nitro, NR_4R_5, or -NHCO(C_1-C_4 alkyl);

R_2 is hydrogen, CH_3CO-, NH_2, or hydroxy;

R_3 is hydrogen, $(CH_2)_m$aryl, C_1-C_4 alkyl, -COO(C_1-C_4 alkyl), -$CONR_4R_5$, -(C=NH)NH_2, -SO(C_1-C_4 alkyl), -$SO_2(NR_4R_5)$, or -SO_2(C_1-C_4 alkyl);

R_4 and R_5 are independently hydrogen, C_1-C_4 alkyl, phenyl, benzyl, or combine to the nitrogen to which they are bonded to form a saturated or unsaturated 5 or 6 member ring;

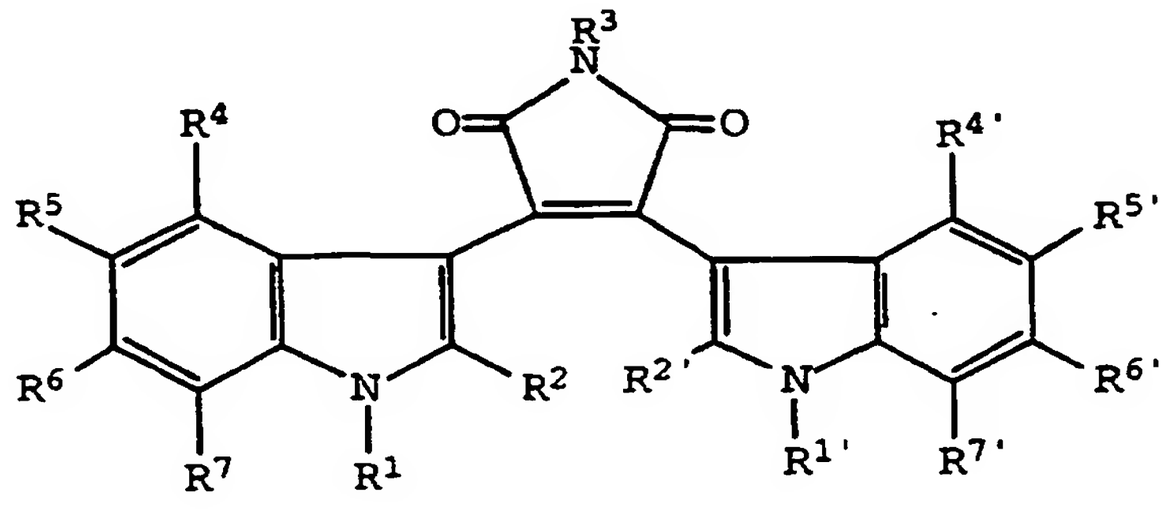
AA is an amino acid residue;

m is independently 0, 1, 2, or 3; and

n is independently 2, 3, 4, or 5,

or a pharmaceutically acceptable salt thereof, and an antioxidant, essential fatty acid or prostacyclin agent or a pharmaceutically acceptable salt thereof.

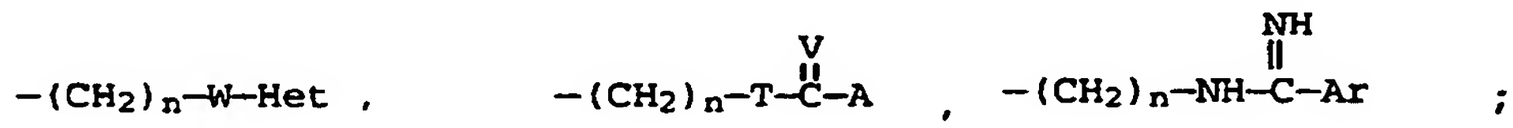
Another preferred embodiment of the present invention provides compositions comprising compounds of Formula II:



(II)

wherein:

R^1 and $R^{1'}$ are independently hydrogen, alkyl, haloalkyl, alkenyl, arylalkyl, alkoxyalkyl, hydroxyalkyl, aminoalkyl, monoalkylaminoalkyl, dialkylaminoalkyl, acylaminoalkyl, acyloxyalkyl, cyanoalkyl, amidinoalkyl, carboxyalkyl, alkoxycarbonylalkyl, aminocarbonylalkyl, or a group of the formula:



(a) (b) (c)

Het signifies a heterocyclyl group;

W signifies NH, S or a bond;

T signifies NH or S;

V signifies O, S, NH, or NCN;

A signifies alkylthio, amino, monoalkylamino or dialkylamino;

Ar signifies aryl;

R^2 and $R^{2'}$ are independently hydrogen, alkyl, alkoxyalkyl, hydroxyalkyl, C_1-C_3 alkylthio, $S(0)C_1$-C_3 alkyl, CF_3; or R^1 and R^2 can combine to form $-(CH_2)_r-X-CH_2-$;

R^3 is hydrogen or CH_3CO;

R^4, $R^{4'}$, R^5, $R^{5'}$, R^6, $R^{6'}$, R^7 and $R^{7'}$ are independently hydrogen, halogen, alkyl, hydroxy, alkoxy, -$COO(C_1$-C_3 alkyl), CF_3, nitro, amino, acetylamino, monoalkylamino, dialkylamino, alkylthio, C_1-C_3 alkylthio, or $S(0)C_1$-C_3 alkyl;

X is CHR^8 or NR^8;

R^8 is $(CH_2)_sR^9$;

R^9 is hydrogen, hydroxy, alkoxy, amino, monoalkylamino, dialkylamino, trialkylamino, azido, acylamino, alkoxycarbonyl, cyano, amidino, or aminocarbonyl;

n is 1, 2, 3, 4, 5 or 6;

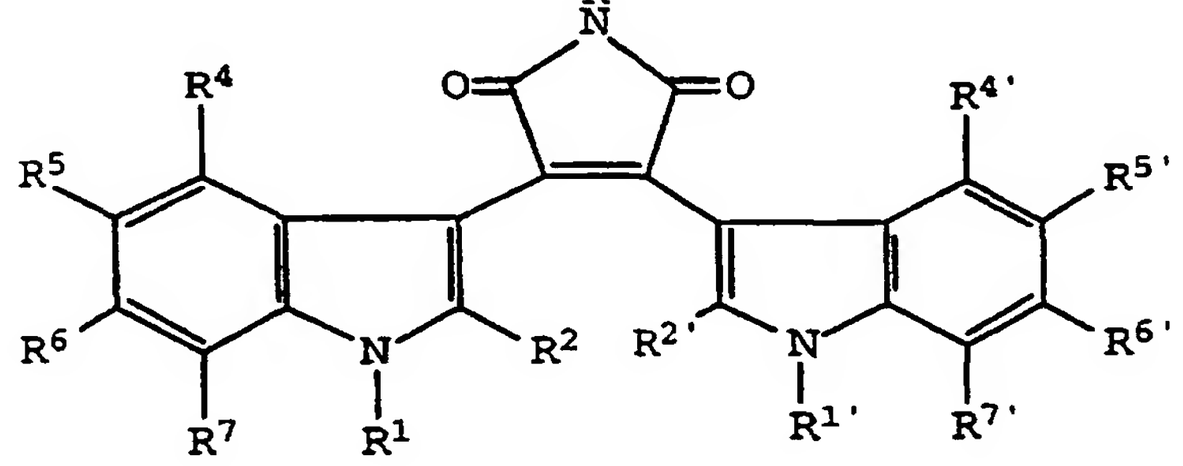
r is 1, 2, or 3; and

s is 0, 1, 2 or 3,

or a pharmaceutically acceptable salt thereof,

and an antioxidant, essential fatty acid or prostacyclin agent or a pharmaceutically acceptable salt thereof.

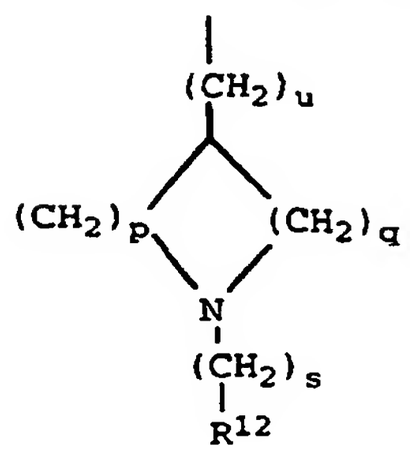
Another preferred embodiment of the present invention provides compositions comprising a compound of Formula III



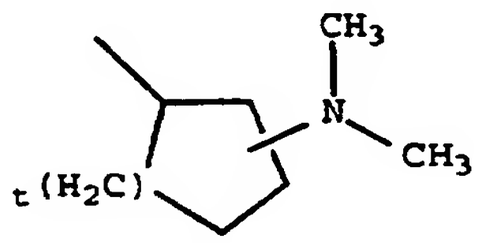
(III)

wherein:

R^1 is

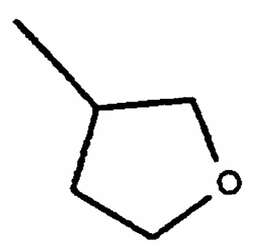


(d)



(e)

or



(f);

$R^{1'}$ is hydrogen, C_1-C_4 alkyl, aminoalkyl, monoalkylaminoalkyl, or dialkylaminoalkyl;

R^2 and $R^{2'}$ are independently hydrogen, alkyl, alkoxyalkyl, hydroxyalkyl, C_1-C_3 alkylthio, $S(0)C_1$-C_3 alkyl, CF_3;

R^3 is hydrogen or CH_3CO-;

R^4, $R^{4'}$, R^5, $R^{5'}$, R^6, $R^{6'}$, R^7 and $R^{7'}$ are independently hydrogen, halogen, alkyl, hydroxy, alkoxy, -$COO(C_1$-C_3 alkyl), CF_3, nitro, amino, acetylamino, monoalkylamino, dialkylamino, alkylthio, C_1-C_3 alkylthio, or $S(0)C_1$-C_3 alkyl;

R^{12} is hydrogen, alkyl, haloalkyl, cycloalkyl, acetyl, aryl, -CH(aryl)$_2$, amino, monoalkylamino, dialkylamino, guanidino, -C(=N(alkoxycarbonyl))NH(alkyoxycarbonyl), amidino, hydroxy, carboxy, alkoxycarbonyl or heterocyclyl;

p and q are independently 1, 2, 3, or 4;

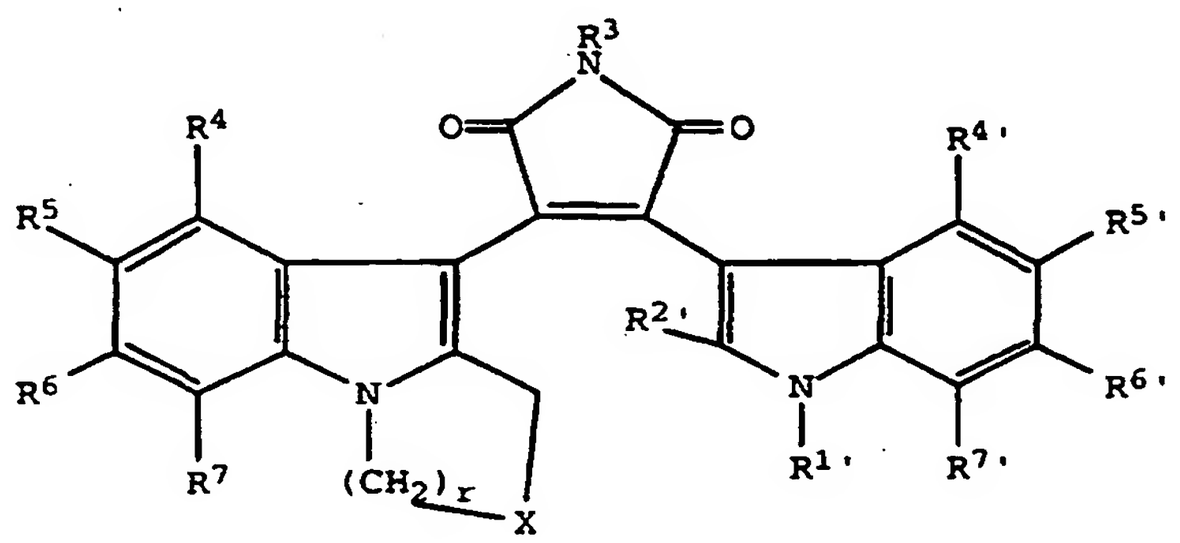
s is 0, 1, 2 or 3;

t is 1 or 2;

u is 0 or 1; or

a pharmaceutically acceptable salt thereof, and an antioxidant, essential fatty acid or prostacyclin agent or a pharmaceutically acceptable salt thereof.

Another preferred embodiment of the present invention provides for compositions comprising a compound of Formula IV



(IV)

wherein:

$R^{1'}$ is hydrogen, C_1-C_4 alkyl, aminoalkyl, monoalkylaminoalkyl, or dialkylaminoalkyl;

$R^{2'}$ is hydrogen, alkyl, alkoxyalkyl, hydroxyalkyl, C_1-C_3 alkylthio, S(0)C_1-C_3 alkyl, CF_3;

R^3 is hydrogen or CH_3CO-;

R^4, $R^{4'}$, R^5, $R^{5'}$, R^6, $R^{6'}$, R^7 and $R^{7'}$ are independently hydrogen, halogen, alkyl, hydroxy, alkoxy, -COO(C_1-C_3 alkyl), CF_3, nitro, amino, acetylamino, monoalkylamino, dialkylamino, alkylthio, C_1-C_3 alkylthio, or S(0)C_1-C_3 alkyl;

X is CR^8R^9;

R^8 is $(CH_2)_sR^{10}$;

R^9 is $(CH_2)_sR^{11}$;

R^{10} and R^{11} are independently hydroxy, alkoxy, carboxy, acyloxy, amino, monoalkylamino, dialkylamino, trialkylamino, azido, acylamino, alkoxycarbonyl, cyano, amidino, or aminocarbonyl;

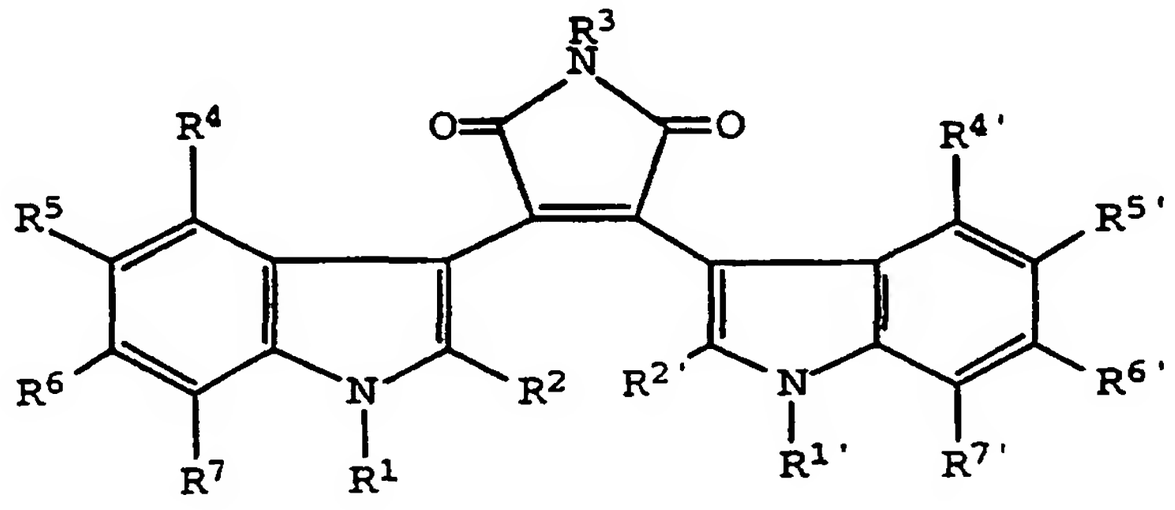
r is 1, 2, or 3;

s is 0, 1, 2 or 3; or

a pharmaceutically acceptable salt thereof,

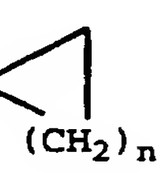
and an antioxidant, essential fatty acid or prostacyclin agent or pharmaceutically acceptable salt thereof.

Another preferred embodiment of the present invention provides compositions comprising a compound of Formula V



(V)

wherein:

R^1 is $-(CH_2)_n-O-\overset{O}{\overset{\|}{C}}-NH\text{-cycloalkyl}$, $-C_1\text{-}C_4$ alkyl-NH-  or alkylglycose residue;

$R^{1'}$ is hydrogen, $C_1\text{-}C_4$ alkyl, cyclopropylmethyl, aminoalkyl, monoalkylaminoalkyl, or dialkylaminoalkyl;

R^2 and $R^{2'}$ are independently hydrogen, alkyl, alkoxyalkyl, hydroxyalkyl, $C_1\text{-}C_3$ alkylthio, $S(0)C_1\text{-}C_3$ alkyl, CF_3;

R^3 is hydrogen or CH_3CO-;

R^4, $R^{4'}$, R^5, $R^{5'}$, R^6, $R^{6'}$, R^7 and $R^{7'}$ are independently hydrogen, halogen, alkyl, hydroxy, alkoxy, -COO(C_1-C_3 alkyl), CF_3, nitro, amino, acetylamino, monoalkylamino, dialkylamino, alkylthio, C_1-C_3 alkylthio, or S(0)C_1-C_3 alkyl;

n is 1, 2, 3, 4, 5 or 6; or

a pharmaceutically acceptable salt thereof, and an antioxidant, essential fatty acid or prostacyclin agent or pharmaceutically acceptable salt thereof.

As used herein, the following definitions apply.

The term "antioxidant" represents lipophilic free radical scavengers, hydrophilic free radical scavengers, and transition metal chelators. Examples of lipophilic free radical scavengers include vitamin E, probucol, butylated hydroxytoluene, beta-carotene, lycopene, flavenoids (for example silybin, diosmin, hesperidin and delphinidin), ubiquinol (coenzyme Q), and lazaroids (for example, tirilazad and 21-aminosteroids). Examples of hydrophilic free radical scavengers include lipoic acid, vitamin C, superoxide dismutase, dihydrolipoic acid, acetyl-L-cysteine, glutathione, and dimethylthiourea. Examples of transition metal chelators include deferoxamine and trientine. Other antioxidants include aminoguanidine and it's derivatives.

The term "essential fatty acid" represents n-6 agents downstream of linoleic acid in the metabolic pathway but upstream of the formation of prostanoids by cyclooxygenase. Examples of essential fatty acids include γ-linolenic acid and arachidonic acid.

The term "prostacyclin agent" represents agents which improve prostacyclin production or potentiate prostacyclin action (for example type III phosphodiesterase inhibitors, for example cilostazol), or act as prostacyclin analogues (for example, iloprost, beraprost sodium).

The term "pharmaceutically effective amount", as used herein, represents an amount of a composition of the

invention, or amount of each of the components being co-administered in a method of treatment of the invention, that is capable of inhibiting PKC activity in mammals. The particular dose of the compound or compounds administered according to this invention will, of course, be determined by the particular circumstances surrounding the case, including the compound administered, the route of administration, the particular condition being treated, and similar considerations. The compounds can be administered by a variety of routes including the oral, rectal, transdermal, subcutaneous, topical, intravenous, intramuscular or intranasal routes. For all indications, a typical daily dose will contain from about 0.01 mg/kg to about 20 mg/kg of the PKC inhibiting compound, and about 0.01 mg/kg to about 500 mg/kg of the antioxidant, essential fatty acid or prostacyclin agent. However, for topical administration a typical dosage is about 1 to about 500 µg of the PKC inhibiting compound per cm^2 of an affected tissue.

The term "treating," as used herein, describes the management and care of a patient for the purpose of combating the disease, condition, or disorder and includes the administration of a composition of the present invention, or a method of treatment of the present invention to prevent the onset of the symptoms or complications, alleviating the symptoms or complications, or eliminating the disease, condition, or disorder.

The term "isozyme selective" means the preferential inhibition of protein kinase C beta-1 or beta-2 isozyme over protein kinase C isozymes, alpha, gamma, delta, epsilon, zeta, and eta. In general, the compounds of the preferred compositions of the present invention demonstrate a minimum of a eight fold differential (preferably a ten fold differential) in the dosage required to inhibit PKC beta-1 or beta-2 isozyme and the dosage required for equal inhibition of the alpha protein kinase C isozyme as measured in the PKC assay. The compounds demonstrate this

differential across the range of inhibition and are exemplified at the IC_{50}, i.e., a 50% inhibition. Thus, isozyme-selective compounds inhibit the beta-1 and beta-2 isozymes of protein kinase C at much lower concentrations with lower toxicity by virtue of their minimal inhibition of the other PKC isozymes.

In a preferred embodiment of the present invention, the ratio of the amount of the inhibitor of protein kinase C to the amount of antioxidant, essential fatty acid or prostacyclin agent comprising the pharmaceutical compositions of the present invention, or of the amounts administered in the methods of treatment of the present invention is from 100 to 1, to 1 to 100.

In a more preferred embodiment of the present invention, the ratio of the amount of the inhibitor of protein kinase C to the amount of antioxidant, essential fatty acid or prostacyclin agent comprising the pharmaceutical compositions of the present invention, or of the amounts administered in the methods of treatment of the present invention is from 10 to 1, to 1 to 10.

For the purposes of the compounds of Formula I, as used herein, the following definitions apply.

The term "halo" represents fluorine, chlorine, bromine, or iodine.

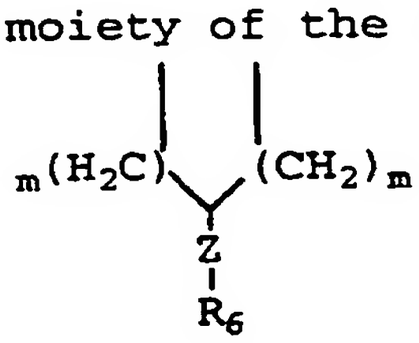
The term "C_1-C_4 alkyl" represents a cyclo, straight or branched chain alkyl group having from one to four carbon atoms such as methyl, ethyl, n-propyl, isopropyl, cyclopropyl, n-butyl, isobutyl, sec-butyl, t-butyl and the like. A haloalkyl is one such alkyl substituted with one or more halo atoms, preferably one to three halo atoms. An example of a haloalkyl is trifluoromethyl. A C_1-C_4 alkoxy is a C_1-C_4 alkyl group covalently bonded by an -O- linkage.

The term "C_1-C_4 alkylene" represents a one to four carbon, straight alkylene moiety of the formula -$(CH_2)_r$- wherein r is one to four. Examples of C_1-C_4 alkylene include methylene, ethylene, trimethylene, methylethylene, tetramethylene, and the like. Similarly, a "C_2-C_6 alkylene" represents a two to six carbon, straight alkylene moiety. Preferably, C_2-C_6 alkylene is a two to four carbon alkylene.

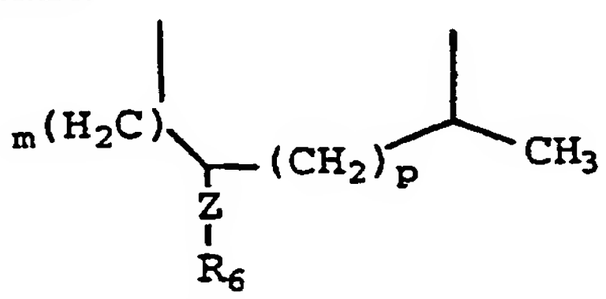
The term "C_2-C_6 alkenylene" represents a two to six carbon, straight or branched hydrocarbon containing one or more double bonds, preferably one or two double bonds. Examples of a C_2-C_6 alkenylene include ethenylene, propenylene, 1,3 butadieneyl, and 1,3,5-hexatrienyl.

The term "aryl" represents a substituted or unsubstituted phenyl or naphthyl. Aryl may be optionally substituted with one or two groups independently selected from hydroxy, carboxy, C_1-C_4 alkoxy, C_1-C_4 alkyl, haloalkyl, nitro, -NR_4R_5, -NHCO(C_1-C_4 alkyl), -NHCO(benzyl), -NHCO(phenyl), SH, S(C_1-C_4 alkyl), -OCO(C_1-C_4 alkyl), -$SO_2(NR_4R_5)$, -SO_2(C_1-C_4 alkyl), -SO_2(phenyl), or halo. The term $(CH_2)_m$aryl is preferably benzyl or phenyl.

The term "substituted alkylene" represents a moiety of the formula:



or



wherein Z is -$(CH_2)_p$- or -$(CH_2)_p$-O-$(CH_2)_p$-; R_6 is C_1-C_4 alkyl, C_1-C_4 alkoxy, $(CH_2)_m$aryl, $(CH_2)_m$aryloxy, hydroxy, carboxy, -COO(C_1-C_4 alkyl)), -COO(($CH_2)_m$aryl), -CO(C_1-C_4 alkyl), -NR_4R_5, NH(CF_3), -N(CF_3)(CH_3), -N(R_4R_5)(OR_5), -NH$(CH_2)_m$aryl, -NH$(CH_2)_m$pyridyl, -CONH(($CH_2)_m$aryl), -CONH(C_1-C_4 alkyl), -NHCO(C_1-C_4 alkyl), -NHCO$(CH_2)_m$aryl, -OCONH(C_1-C_4 alkyl), -OCONH$(CH_2)_m$aryl, -NHCOO(alkyl), -NHCOO(benzyl), -$NHSO_2$(C_1-C_4 alkyl), -$NHSO_2(CH_2)_m$aryl, -CN, -SH, -S(C_1-C_4 alkyl), -S(aryl), -$SO_2(NR_4R_5)$, -SO_2(C_1-C_4 alkyl), -SO(C_1-C_4 alkyl), glycosyl, or heterocycle; R_4 and R_5 are independently

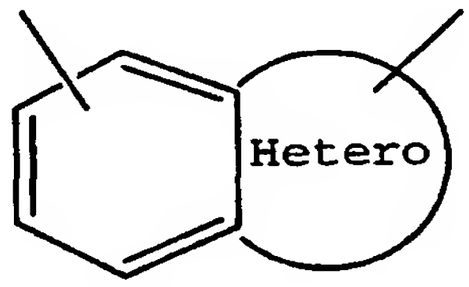
hydrogen, C_1-C_4 alkyl, phenyl, benzyl, or combine to the nitrogen to which they are bonded to form a saturated or unsaturated 5 or 6 member ring; p is independently 0, 1 or 2; and m is independently 0, 1, 2, or 3. Preferably Z is -CH_2-; and R_6 is C_1-C_4 alkyl, aryl, or -NR_4R_5.

The term "heterocycle" represents a stable, substituted or unsubstituted, saturated or unsaturated 5 or 6 membered ring, said ring having from one to four heteroatoms that are the same or different and that are selected from the group consisting of sulfur, oxygen, and nitrogen; and when heterocycle contains two adjacent carbon atoms, the adjacent carbon atoms may be structured to form a group of the formula -CH=CH-; provided that (1) when the heterocyclic ring contains 5 members, the heteroatoms comprise not more than two sulfur or two oxygen atoms but not both; and (2) when the heterocyclic ring contains 6 members and is aromatic, sulfur and oxygen are not present. The heterocycle may be attached at any carbon or nitrogen which affords a stable structure. The heterocycle may be substituted with one or two groups independently selected from C_1-C_4 alkyl, C_1-C_4 alkoxy, hydroxy, acetyl, carboxy, haloalkyl, nitro, -NR_4R_5, -NHCO(C_1-C_4 alkyl), -NHCO(benzyl), -NHCO(phenyl), SH, S(C_1-C_4 alkyl), -OCO(C_1-C_4 alkyl), -SO_2(NR_4R_5), -SO_2(C_1-C_4 alkyl), -SO_2(phenyl), or halo. Examples of an heterocycle include pyrazole, pyrazoline, imidazole, acetylimidazole, isoxazole, triazole, tetrazole, oxazole, 1,3-dioxolone, thiazole, oxadiazole, thiadiazole, pyridine, dipyridyl, pyrimidine, piperizine, morpholine, pyrazine, pyrrolidine, piperidine, piperazine, oxazolidinone, imidozolidinone, and aminopyridine.

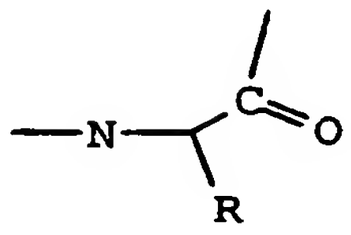
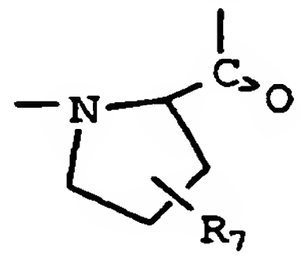
The term "glycosyl" represents a 5 or 6 carbon sugars, preferably selected from allosyl, altrosyl, glucosyl, mannosyl, gulosyl, idosyl, galactosyl, talosyl, arabinosyl, xylosyl, lyxosyl, rhamnosyl, ribosyl, deoxyfuranosyl, deoxypyranosyl, and deoxyribosyl. The glycose may be azide substituted, O-acetylated, O-

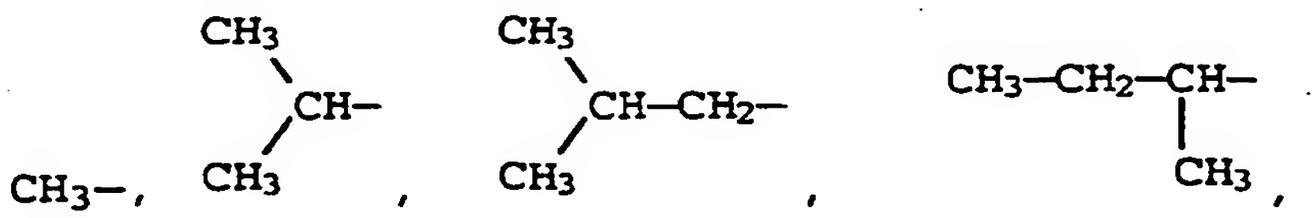
methylated, amino, mono, and di-alkylamino substituted, or acylamino substituted.

The term "fused bicyclic" represents a stable fused bicyclic ring system of the formula:

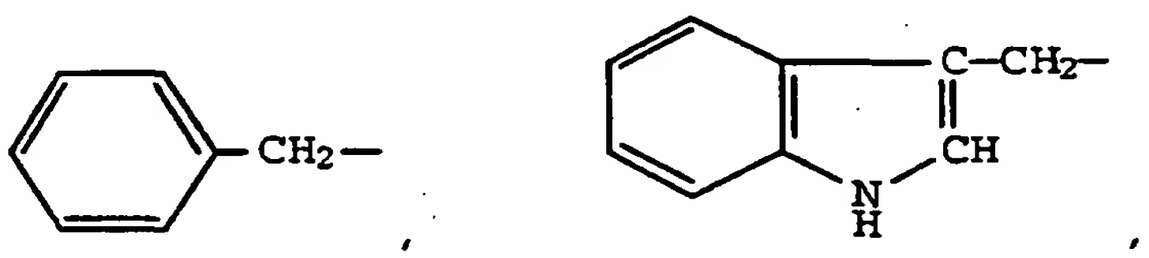


wherein Hetero represents a substituted or unsubstituted, saturated or unsaturated 5 or 6 membered ring, said ring having from one to three heteroatoms that are the same or different and that are selected from the group consisting of sulfur, oxygen, and nitrogen; and when Hetero contains two adjacent carbon atoms, the adjacent carbon atoms may be structured to form a group of the formula -CH=CH-; provided that (1) when the Hetero ring contains 5 members, the heteroatoms comprise not more than two sulfur or two oxygen atoms but not both; and (2) when the Hetero ring contains 6 members and is aromatic, sulfur and oxygen are not present. The fused bicyclic may be attached at any carbon or nitrogen atom which affords a stable structure. The fused bicyclic may be substituted with one or two groups independently selected from C_1-C_4 alkyl, C_1-C_4 alkoxy, hydroxy, carboxy, haloalkyl, nitro, -NR_4R_5, -NHCO(C_1-C_4 alkyl), -NHCO(benzyl), -NHCO(phenyl), SH, S(C_1-C_4 alkyl), -OCO(C_1-C_4 alkyl), -SO_2(NR_4R_5), -SO_2(C_1-C_4 alkyl), -SO_2(phenyl), or halo. Examples of a fused bicyclic include indole, imidazo(1,2-a)pyridine, benzotriazole, benzimidazole, benzotriazole, benzoxazole, benzoxathiazole, quinoline, isoquinoline, phthalazine, quinazoline, quinazolinone, quinoxaline, and aminoisoquinoline.

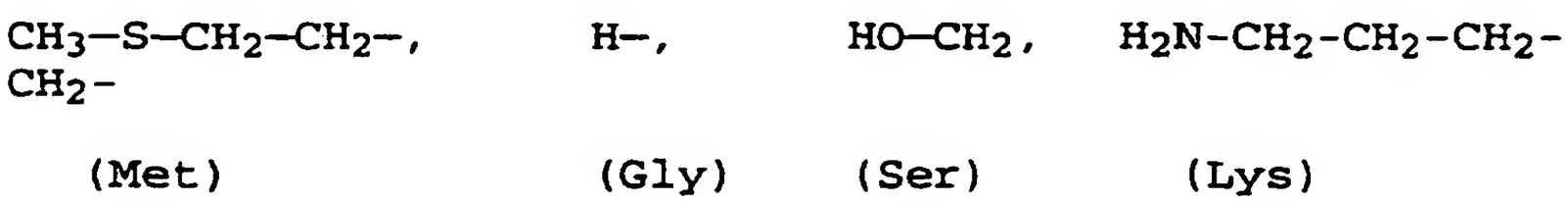
The term "amino acid residue" refers to moiety of the formula  or  wherein R represents the variable side chain of an amino acid and R7 is hydrogen or hydroxy. The variable side chain of an amino acid represents the atom or group bonded to an α-carbon atom also having bonded thereto a carboxyl and an amino group. For example, the variable region of the naturally occurring amino acids are of the formulas:



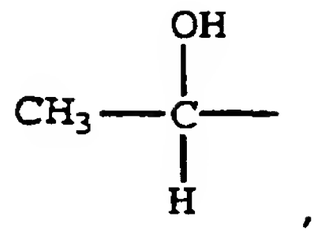
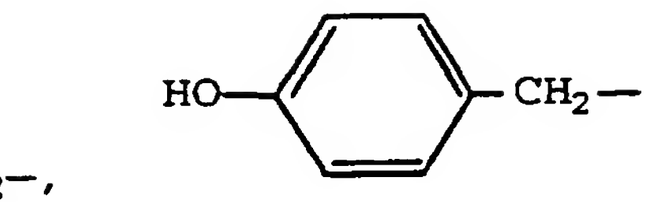
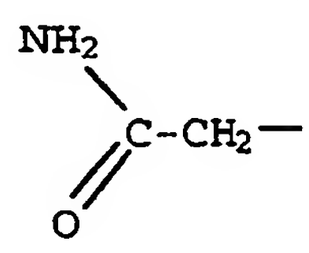
(Ala) (Val) (Leu) (Ile)



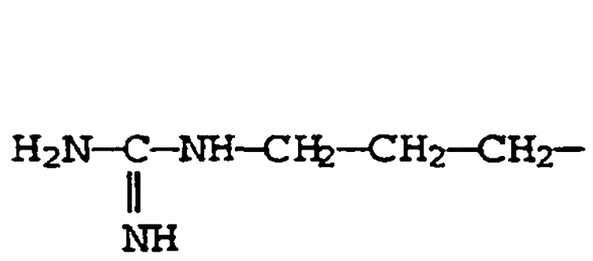
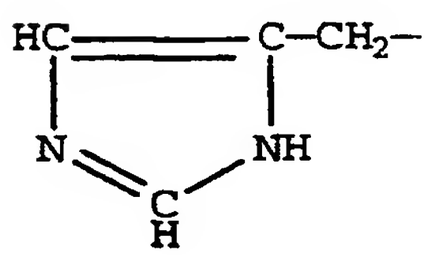
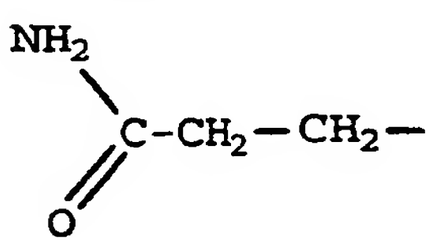
(Phe) (Trp)



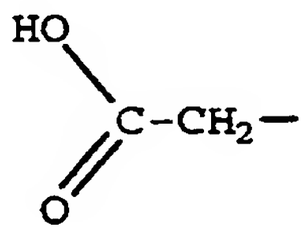
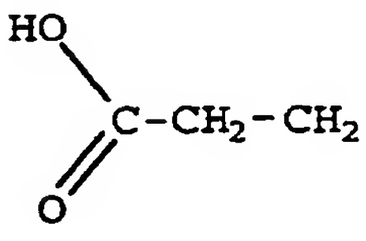
(Met) (Gly) (Ser) (Lys)

(Thr) (Cys) (Tyr) (Asn)

(Arg) (His) (Gln)

(Asp) (Glu)

In addition to the naturally occurring amino acids, the term amino acid residue includes positional isomers and variants. Examples of positional isomers and variants represented by amino acid residue include: 2-Aminoadipic acid (Aad), 3-aminoadipic acid (bAad), β-alanine (bAla), 2-aminobutyric acid (Abu), 4-aminobutyric acid (4Abu), 6-aminocaproic acid (Acp), 2-aminoheptanoic acid (Ahe), 2-aminoisobutyric acid (Aib), 3-aminoisobutyric acid (bAib), 2-aminopimelic acid (Apm), 2,4-diaminobutyric acid (Dbu), desmosine (Des), 2,2'-

diaminopimelic acid (Dpm), 2,3-diaminopropionic acid (Dpr), N-ethylglycine (EtGly), N-ethylasparagine (EtAsn), hydroxylysine (Hyl), allohydroxylysine (aHyl), 3-hydroxyproline (3Hyp), 4-hydroxyproline (4Hyp), isodesmosine (Ide), allo-isoleucine (alle), naphthylglycine, N-methylglycine (MeGly), N-methylisoleucine (MeIle), N-methyllysine (MeLys), norvaline (Nva), norleucine (Nle), ornithine (Orn), phenylglycine, cyanoalanine (CA), γ-carboxyglutamate, O-phosphoserine, α-naphthylalanine (NA), β-naphthylalanine (bNA), S-galactosyl cysteine, glycinamide, N-formylmethionine, tyrosine-O-sulfate and the like. These amino acid residues may be in either the D or L configuration. Unless otherwise specified, a reference to an amino acid will refer to the L configuration.

For the purposes of the compounds of Formulas II, III, IV and V, as used herein, the following definitions apply:

The term "alkyl", alone or in combinations, means a straight or branched-chain alkyl group containing from one to seven, preferably one to four, carbon atoms such as methyl, ethyl, propyl, isopropyl, butyl, sec-butyl, t-butyl and pentyl. The term "C_1-C_4 alkyl" is an alkyl limited to one to four carbon atoms.

The term "cycloalkyl", alone or in combinations, means a three to seven carbon cycloalkyl, for example, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, and cycloheptyl.

The term "alkenyl" means a two to seven carbon, straight or branched hydrocarbon containing one or more double bonds, preferably one or two double bonds. Examples of alkenyl include ethenylene, propenylene, 1,3 butadienyl, and 1,3,5-hexatrienyl.

The term "alkoxy", alone or in combinations, is an alkyl covalently bonded by an -O- linkage. Examples of alkoxy groups are methoxy, ethoxy, propoxy, isopropoxy, butoxy and t-butoxy. An alkoxyalkyl is, for example,

$CH_3(CH_2)$-O-$(CH_2)_m$- wherein m is the from one to seven or preferably one to four. The term alkoxycarbonyl is, for example, t-butoxycarbonyl or BOC.

A haloalkyl group is an alkyl with one or more, preferably one to three halogen atoms, examples of such group CH_2Cl, CF_3, CH_2CF_3, $CH_2(CF_2)_2CF_3$, and the like.

The acyl moiety of an acylamino or acylaminoalkyl group is derived from an alkanoic acid containing a maximum of 7, preferably a maximum of 4, carbon atoms (e.g. acetyl, propionyl or butyryl) or from an aromatic carboxylic acid (e.g. benzoyl). An acyloxy is one such acyl bonded by an -O- linkage, for example, acetyloxy, $CH_3C(=O)O$-. An acylamino is, for example, $CH_3(C=O)NH$- (acetylamino). Likewise, an acylaminoalkyl is $CH_3(C=O)NH(CH_2)_m$-.

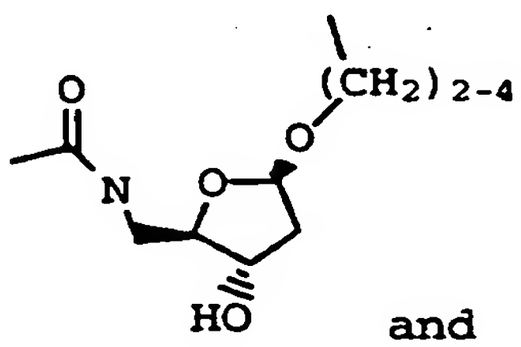
The term "aryl", alone or in combinations means an unsubstituted phenyl group or a phenyl group carrying one or more, preferably one to three, substituents, independently selected from halogen, alkyl, hydroxy, benzyloxy, alkoxy, haloalkyl, nitro, amino, acylamino, monoalkylamino, dialkylamino, alkylthio, alkylsulfinyl, alkylsulfonyl and cyano. The term arylalkyl is preferably benzyl.

The term "halogen" means fluorine, chlorine, bromine or iodine.

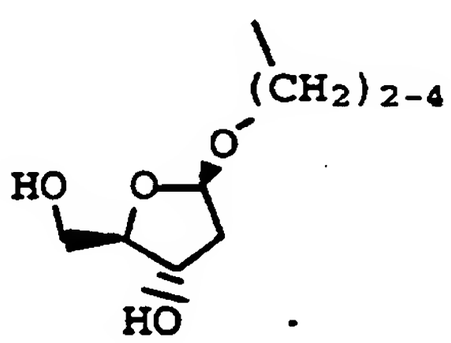
The heterocyclic group denoted by "Het" or "heterocyclyl" can be a stable, saturated, partially unsaturated, or aromatic 5- or 6-membered heterocyclic group. The heterocyclic ring consists of carbon atoms and from one to three heteroatoms independently selected from the group consisting of nitrogen, oxygen, and sulfur. The heterocyclic group can be optionally substituted with one to three substituents independently selected from halogen, alkyl, hydroxy, alkoxy, haloalkyl, nitro, amino, acylamino, monoalkylamino, dialkylamino, alkylthio, alkylsulfinyl and alkylsulfonyl or, when the heterocyclyl group is an aromatic nitrogen-containing heterocyclic group, the nitrogen atom can carry an oxide group. Examples of such heterocyclyl

groups are imidazolyl, imidazolinyl, thiazolinyl, pyridyl, indolyl, furyl, and pyrimidinyl.

The term "alkylglycose residue" represents a glycose moiety linked in the C-1 position to the indolyl via a C_2-C_4 alkyl. Glycoses included in alkylglycose residue are natural or unnatural 5 or 6 carbon sugars, preferably selected from allosyl, altrosyl, glucosyl, mannosyl, gulosyl, idosyl, galactosyl, talosyl, arabinosyl, xylosyl, lyxosyl, rhamnosyl, ribosyl, deoxyfuranosyl, deoxypyranosyl, and deoxyribosyl. The glycose may be azide substituted, O-acetylated, O-methylated, amino, mono, and di-alkylamino substituted, or acylamino substituted. For example, alkylglycose residue includes:



and



By virtue of their acidic moieties, the compounds of Formulas I to V, antioxidants, essential fatty acids, or prostacyclin agents include the pharmaceutically acceptable base addition salts thereof. Such salts include those derived from inorganic bases such as ammonium and alkali and alkaline earth metal hydroxides, carbonates, bicarbonates, and the like, as well as salts derived from basic organic amines such as aliphatic and aromatic amines, aliphatic diamines, hydroxy alkamines, and the like. Such bases useful in preparing the salts of this invention thus include ammonium hydroxide, potassium carbonate, sodium bicarbonate, calcium hydroxide, methylamine, diethylamine, ethylenediamine, cyclohexylamine, ethanolamine and the like.

Because of the basic moiety, the compounds of Formulas I to V, antioxidants, essential fatty acids, or prostacyclin agents can also exist as pharmaceutically

acceptable acid addition salts. Acids commonly employed to form such salts include inorganic acids such as hydrochloric, hydrobromic, hydroiodic, sulfuric and phosphoric acid, as well as organic acids such as para-toluenesulfonic, methanesulfonic, oxalic, para-bromophenylsulfonic, carbonic, succinic, citric, benzoic, acetic acid, and related inorganic and organic acids. Such pharmaceutically acceptable salts thus include sulfate, pyrosulfate, bisulfate, sulfite, bisulfite, phosphate, monohydrogenphosphate, dihydrogenphosphate, metaphosphate, pyrophosphate, chloride, bromide, iodide, acetate, propionate, decanoate, caprylate, acrylate, formate, isobutyrate, heptanoate, propiolate, oxalate, malonate, succinate, suberate, sebacate, fumarate, maleate, 2-butyne-1,4 dioate, 3-hexyne-2, 5-dioate, benzoate, chlorobenzoate, hydroxybenzoate, methoxybenzoate, phthalate, xylenesulfonate, phenylacetate, phenylpropionate, phenylbutyrate, citrate, lactate, hippurate, β-hydroxybutyrate, glycollate, maleate, tartrate, methanesulfonate, propanesulfonate, naphthalene-1-sulfonate, naphthalene-2-sulfonate, mandelate and the like salts.

The pharmaceutically acceptable salts of the compounds of Formulas I to V, antioxidants, essential fatty acids, or prostacyclin agents can also exist as various solvates, such as with water, methanol, ethanol, dimethylformamide, ethyl acetate and the like. Mixtures of such solvates can also be prepared. The source of such solvate can be from the solvent of crystallization, inherent in the solvent of preparation or crystallization, or adventitious to such solvent. Such solvates are within the scope of the present invention.

It is recognized that various stereoisomeric forms of the compounds of Formulas I to V may exist. The compounds are normally prepared as racemates and can conveniently be used as such, but individual enantiomers can be isolated or synthesized by conventional techniques if so

desired. Such racemates and individual enantiomers and mixtures thereof form part of the present invention.

The invention also encompasses compositions and methods of treatment comprising the pharmaceutically acceptable prodrugs of the compounds of Formulas I to V. A prodrug is a drug which has been chemically modified and may be biologically inactive at its site of action, but which may be degraded or modified by one or more enzymatic or other in vivo processes to the parent bioactive form. This prodrug should have a different pharmacokinetic profile than the parent, enabling easier absorption across the mucosal epithelium, better salt formation or solubility, and/or improved systemic stability (an increase in plasma half-life, for example). Typically, such chemical modifications include the following:

1) ester or amide derivatives which may be cleaved by esterases or lipases;

2) peptides which may be recognized by specific or nonspecific proteases; or

3) derivatives that accumulate at a site of action through membrane selection of a prodrug form or a modified prodrug form; or any combination of 1 to 3, supra. Conventional procedures for the selection and preparation of suitable prodrug derivatives are described, for example, in H, Bundgaard, Design of Prodrugs, (1985).

The synthesis of certain bis-indole-N-maleimide derivatives is described in Davis et al. U.S. Patent 5,057,614, herein incorporated by reference.

The syntheses of the PKC inhibitors which comprise the preferred compositions, co-treatments, and methods of treatment of the present invention are described, for example, in the U.S. Patents incorporated herein by reference as discussed hereinabove. For example, the compounds of Formula I are so described in U.S. Patent No. 5,552,396. The compounds of Formulas II, III, IV and V are so described in U.S. Patent Nos. 5,545,636, 5,661,173, 5,663,152 and 5,672,618.

As previously noted, the compounds comprising the compositions, co-treatments and methods of treatment of the present invention are potent, protein kinase C inhibitors. The compounds are selective for protein kinase C over other kinases.

The ability of the PKC-inhibiting compounds comprising the preferred compositions and methods of treatment of the present invention to selectively inhibit protein kinase C was determined in the Calcium Calmodulin Dependent Protein Kinase Assay, Casein Protein Kinase II assay, cAMP-Dependent Protein Kinase Catalytic Subunit assay and the Protein-Tyrosine Kinase assay, as described, for example in U.S. Patent No. 5,624,949, incorporated herein by reference, in the PKC enzyme assay as described, for example in U.S. Patents Nos. 5,545,636 and 5,668,152, incorporated herein by reference.

Increased protein kinase C (PKC) activation by elevated diacyldiglycerol (DAG) occurs in several vascular tissues in experimental dibetes. For retina and kidney this has been linked to early complications, which were attenuated by treatment with a β-isoform specific PKC antagonist, (S)-3,4-[(N,N'-1,1'-((2''-ethoxy)-3'''(O)-4'''(N,N-dimethylamino)-butane)-bis-(3,3'-indolyl)]-1(H)pyrrole-2,5-dione (LY333531) (Eli Lilly and Company, Indianapolis, Indiana, USA). There is also a marked vascular component in the etiology of experimental neuropathy. Therefore, the efficacy of LY333531 against conduction velocity (NCV) deficits was determined. Further characterized was the diabetic vascular deficit and the role of PKCβ in resistance vessel responses, using the mesenteric vascular bed. Furthermore, vitamin E, as well as having antioxidant properties that could benefit neurovascular function, can modulate PKC indirectly through effects on DAG kinase activity. Therefore the interaction between LY333531 and vitamin E treatment was examined. After 6 weeks of untreated streptozotocin-diabetes, 2 weeks of oral treatment with LY333531 dose dependently corrected 19.7±0.8% (±SEM;

p<0.001) sciatic motor and 13.9±0.7% (p<0.001) saphenous sensory NCV deficits were seen. EC_{50} values being 0.88 and 0.51 mg/kg respectively. At a dose of 10 mg/kg, saphenous sensory NCV was completely corrected and sciatic motor NCV was improved by 91.1±3.3% (p<.001). This dose also completely corrected a 50.4±3.2% (p<.001) diabetic deficit in sciatic endoneurial nutritive blood flow. Acetylcholine (ACh) induced relaxation of phenylephrine-precontracted mesenteric vasculature was diminished by 8 weeks of diabetes (control, maximum relaxation 97.8±0.9%, $-\log EC_{50}$ 7.75±0.14; diabetes, 66.2±4.8%, 7.04±0.07; p<0.001). This was partially prevented by LY333531 (10mg/kg; 86.4±3.2%, 7.51±0.07; p<0.01). N^G-nitro-L-arginine blocked NO-mediated relaxation, unmasking that due to endothelium-derived hyperpolarising factor. ACh maximum relaxation was reduced by diabetes (14.8±3.6% vs. 73.8±4.0%; p<0.001) and LY333531 was protective (45.7±7.2%, p<0.01). Low doses of LY333531 (0.25 mg/kg) and vitamin E (360mg/kg) on their own had modest effects on motor and sensory NCV (<25.5% correction) and nerve blood flow (<19.0% correction). However, with combined treatment, NCV and blood flow were in the nondiabetic range, demonstrating a marked synergistic interaction (p<0.0001 and p = 0.008 respectively). The combined drug effect on NCV was equivalent to a ~20-fold increase in LY333531 dose. Thus, PKCβ is an important mediator of vascular defects in diabetic rats. LY333531's beneficial action on perfusion of nerve, and probably other tissues, depends partly on restoration of multiple endothelial relaxation mechanisms; an effect that is markedly enhanced by vitamin E co-treatment.

Methods

Male Sprague-Dawley rats (Aberdeen University colony) were used, aged 19 weeks at the start of experiments. Diabetes was induced by an intraperitoneal injection of

streptozotocin (Zeneca Pharmaceuticals, Macclesfield, Cheshire, UK) freshly made up in sterile 154 mM NaCl solution (40-45 mg kg^{-1}). Diabetes was verified after 24 hours by the presence of hyperglycemia and glucosuria (Visidex II and Diastix; Ames, Slough, UK) in non-fasted rats. After final experiments, non-fasted plasma glucose concentration was estimated (GOD-Perid method; Boehringer Mannheim, Mannheim, Germany)on samples taken from the carotid artery cannula. Plasma glucose values for all dabetic groups were in the range 40.7±1.5 to 44.8±1.5 mM (mean ± SEM), which was markedly elevated compared to nondiabetic controls (5.8±0.3 mM).

The following groups of rats (n=8-11) were used. Nondiabetic control, diabetic controls untreated for 8 weeks, and single treatment diabetic groups, untreated for the first 6 weeks and then given 2 weeks of dietary supplementaion with either vitamin E (360 mg/kg rat), α-lipoic acid (racemic mixture, 20 mg/kg rat), γ-linolenic acid (20 mg/kg rat) or LY333531 (0.25 mg/kg rat). Vitamin E, α-lipoic, γ-linolenic acid were obtained from Sigma, Poole, Dorset, UK and LY333531 methane sulfonate salt (LY) was obtained from Eli Lilly, Indianapolis, IN. Three further diabetic groups were used to test for interactions between LY333531 treatment and either vitamin E, α-lipoic, γ-linolenic acid treatments at the above mentioned doses.

Motor and Sensory Nerve Conduction

Rats were anesthetized with thiobutabarbital sodium (Zeneca Pharmacetucials, Macclesfield, Cheshire, UK) by intraperitoneal injection (50-100 mg kg^{-1}). The trachea and one carotid artery were cannulated for respiratory aid and systemic blood pressure recording respectively. The core

temperature of the rat was regulated in the range 37 - 38°C using radiant heat and was monitored with a rectal probe. The sciatic nerve was exposed from sciatic notch to knee and nerve temperature was kept at 36-37°C using radiant heat and was monitored with a near-nerve thermocouple probe. Bipolar stimulating electrodes were placed around the nerve at the notch and the knee. A bipolar recording electrode was placed in the tibialis anterior muscle to monitor stimulus-evoked electromyographic activity. A ground electrode was placed on the thigh muscle. Potentials evoked from each stimulating site were averaged 8 times. Motor conduction velocity was calculated by dividing the distance between stimulating electrodes by the average latency difference between the onset of electromyographic potentials evoked from the 2 sites. Sensory conduction velocity was measured in the saphenous nerve of the same limb. The nerve was exposed between groin and ankle, a short (~ 3 mm) segment was dissected free from muscle and connective tissue in the lower calf/ankle region, and a unipolar platinum wire electrode was used to monitor compound action potentials evoked by stimulation of saphenous nerve proximal to the recording site. Compound action potentials in response to stimulation were averaged 16 times and sensory conduction velocity was calculated by dividing interelectrode distance by the latency of the first inflection of the potential.

Sciatic nerve endoneurial blood flow

Endoneurial blood flow was measured in the contralateral limb by microelectrode polarography and hydrogen clearance. Rats were given neuromuscular blockade using d-tubocurarine (Sigma, 2 mg/kg via the carotid cannula) and artificially ventilated. The level of anesthesia was monitored by observing any reaction of blood

pressure to manipulation, and supplementary thiobutabarbital anesthetic was given as necessary. The sciatic nerve was exposed and the skin around the incision was sutured to a metal ring to form a pool which was filled with mineral oil maintained at 37°C by radiant heat. A fine glass-insulated platinum microelectrode, polarized at 250 mV with respect to a subcutaneous reference electrode, was inserted into the sciatic nerve endoneurium between the sciatic notch and the nerve trifurcation above the knee. 10% hydrogen was added to the inspired gas, the proportions of oxygen and nitrogen being adjusted to 20% and 70% respectively. When the hydrogen current supply recorded by the electrode had stabilized, indicating equilibrium with arterial blood, the hydrogen supply was shut off and nitrogen delivery was increased appropriately. Hydrogen clearance was recorded until a stable baseline was reached, which was defined as no systematic decline in electrode current over 5 minutes. This procedure was then repeated at another nerve site. After the experiment, clearance curves were digitized and mono-or bi-exponential curves were fitted to the data by computer using non-linear regression analysis (Inplot, Graphpad, San Diego, CA). The bi-exponential equation used was:

$$y = a \exp(-bx) + c \exp(-dx) + e$$

where y is the electrode hydrogen current (arbitrary units), x is time (minutes), a and c are weighting constants for fast (non-nutritive) and slow (nutritive clearance components respectively, b is the fast component and d is the slow component (ml min^{-1} $100g^{-1}$). Vascular conductance was calculated by dividing blood flow by the mean arterial blood pressure over the recording period for that particular clearance curve. The averages from the two determinations were taken to represent sciatic endoneurial blood flow parameters.

Statistical analysis

Data were subjected to one way analysis of variance. If a statistically significant ($p<0.05$) effect was found, significance was assigned to individual between group comparisons using the Bonferroni multiple comparison test. Logarithmic transformation was used where there were significant between-group variance inequalities. Predicted values for interaction groups were compared with actual results using the one-sample Student t-test.

The results of the above tests are shown in Table I. In the interaction data of table I, Mean±SEM, (*n*) next to group is number of observations unless indicated as different in parentheses next to a measurement. Sciatic motor NCV (m/s); saphenous sensory NCV (m/s); sciatic endoneurial blood flow (BF - ml/min/100g); mean systemic blood pressure (BP - mm Hg); sciatic endoneurial vascular conductance (VC - ml/min/100g/mm Hg). Drugs: vitamin E (E, 360 mg/kg), α-lipoic acid (LA, 20 mg/kg), γ-linolenic acid (G, 20 mg/kg), LY333531 methane sulfonate salt (LY, 0.25 mg/kg) all administered in the diet.

Table I

Group	Motor NCV	Sensory NCV	BF	BP	VC
Control (10)	63.8±0.4	60.0±0.4	17.3±0.9	140±5	0.125± 0.007
Diabetic (10)	51.2±0.5	51.6±0.4	8.9±0.6	113±6	0.080± 0.004
+E (10)	54.0±0.4	54.1±0.4	10.3±0.8	108±7	0.099± 0.008
+LA (10)	53.9± 0.4(11)	54.6± 0.7(8)	10.7±0.7	114±4	0.096± 0.006
+G (10)	53.5±0.4	54.5±0.4	10.6±0.8	111±6	0.096± 0.006
+LY (10)	54.2±0.3	54.5±0.4	11.0±0.6	108±6	0.105± 0.005
+LY +E (10)	61.4±0.4	60.1±0.6	19.5±1.4	109±8	0.192± 0.021
+LY +LA (10)	62.9±0.4	60.7±0.4 (9)	16.1±1.2	84±5	0.195± 0.011
+LY +G	61.9±0.7	60.1±0.7	15.9±0.8	102±6 (10)	0.160± 0.009 (10)

5

Statistical Analysis

Statistics

One way ANOVA followed by Bonferroni's multiple comparison test for a restricted set of comparisons - all vs control, all vs diabetic, + single treatment vs + same single treatment + LY, +LY vs +LY+treatment.

Motor NCV

Control vs Diabetic, +E, +LA, +G or +LY; p<0.001

Control vs +LY+E; p<0.05; Control vs +LY+LA or +LY+G; NS

Diabetic vs +LY; p<0.001; or +LA or +E; p<0.01; or +G; p<0.05

Diabetic vs +LY+LA, +LY+E, or +LY+G; p<0.001

+LY or +LA vs +LY+LA; +LY or +E vs +LY+E; +LY or +G vs +LY+G; P<0.001

Predicted motor NCV values compared to actual values for interaction groups - one sample t-test +LY+E 56.3 m/s p<0.0001; +LY+LA 56.3 m/s p<0.0001; +LY+G 56.0 m/s p<0.0001

Sensory NCV

Control vs Diabetic, +E, +LA, +G or +LY; p<0.001

Control vs +LY+E, +LY+LA or +LY+G; NS

Diabetic vs +LY or +LA or +G; p<0.01; or +E; p<0.05

Diabetic vs +LY+LA, +LY+E, or +LY+G; p<0.001

+LY or +LA vs +LY+LA; +LY or +E vs +LY+E; +LY or +G vs +LY+G; P<0.001

Predicted sensory NCV values compared to actual values for interaction groups

+LY+E 56.4 m/s p=0.0002; +LY+LA 56.4 m/s p<0.0001; +LY+G 56.4 m/s p=0.0003

Blood Flow

Control vs Diabetic, +E, +LA, +G, +LY; p<0.001

Control vs +LY+E, +LY+LA or +LY+G; NS
Diabetic vs +E, +LA, +G or +LY; NS
Diabetic vs +LY+LA, +LY+E, or +LY+G; $p<0.001$
+LY or +E vs +LY+E; $p<0.001$; +LY or +G vs +LY+G; $p<0.01$;
+LA vs +LY+LA; $p<0.001$; +LY vs +LY+LA; $p<0.01$

Mean systemic blood pressure
Control vs +G; $p<0.05$; control vs +LY, +E, +LY+E; $p<0.01$
Control vs +LY+LA or +LY+G; $p<0.001$
Diabetic vs +LY+LA; $p<0.05$
+LA vs +LY+LA; $p<0.05$
All other comparisons NS

Vascular conductance (log transformed data)
Control vs diabetic; $p<0.001$
Control vs +LY+LA; $p<0.001$; control vs +LY+E+; $p<0.01$
Diabetic vs +LY+E, +LY+LA or +LY+G; $p<0.001$
+E vs +LY+E or +LA vs +LY+LA or +G vs +LY+G; $p<0.001$
+LY vs +LY+E or +LY+LA or +LY+G; $p<0.001$
all other comparisons NS

The compounds of Formulas I to V, antioxidants, essential fatty acids and prostacyclin agents, and the compositions of the present invention are preferably formulated prior to administration. Therefore, yet another embodiment of the present invention is a pharmaceutical formulation comprising PKC inhibitors, or compounds of Formulas I to V, and an antioxidant, essential fatty acid, or prostacyclin agent and one or more pharmaceutically acceptable carriers, diluents or excipients.

The present pharmaceutical formulations are prepared by known procedures using well known and readily available ingredients. In making the compositions of the present invention, the active ingredient or ingredients will

usually be mixed with a carrier, or diluted by a carrier, or enclosed within a carrier which may be in the form of a capsule, sachet, paper or other container. When the carrier serves as a diluent, it may be a solid, semisolid or liquid material which acts as a vehicle, excipient or medium for the active ingredient. Thus, the compositions can be in the form of tablets, pills, powders, lozenges, sachets, cachets, elixirs, suspensions, emulsions, solutions, syrups, aerosol (as a solid or in a liquid medium), soft and hard gelatin capsules, suppositories, sterile injectable solutions and sterile packaged powders.

Some examples of suitable carriers, excipients, and diluents include lactose, dextrose, sucrose, sorbitol, mannitol, starches, gum acacia, calcium phosphate, alginates, tragacanth, gelatin, calcium silicate, microcrystalline cellulose, polyvinylpyrrolidone, cellulose, water syrup, methyl cellulose, methyl and propylhydroxybenzoates, talc, magnesium stearate and mineral oil. The formulations can additionally include lubricating agents, wetting agents, emulsifying and suspending agents, preserving agents, sweetening agents or flavoring agents. The compositions of the invention may be formulated so as to provide quick, sustained or delayed release of the active ingredient or ingredients after administration to the patient. The compositions are preferably formulated in a unit dosage form, each dosage containing from about 1 to about 500 mg, more usually about 5 to about 300 mg, of the active ingredient or ingredients. However, it will be understood that the therapeutic dosage administered will be determined by the physician in the light of the relevant circumstances including the condition to be treated, the choice of compound or compounds to be administered and the chosen route of administration, and therefore the above dosage ranges are not intended to limit the scope of the invention in any way. The term "unit dosage form" refers to physically discrete units suitable as unitary dosages for human subjects and other mammals, each unit containing a

predetermined quantity of active material or materials calculated to produce the desired therapeutic effect, in association with a suitable pharmaceutical carrier.

In addition to the above formulations, the compositions of the present invention may be administered topically. Topical formulations are ointments, creams, and gels.

Ointments generally are prepared using either (1) an oleaginous base, i.e., one consisting of fixed oils or hydrocarbons, such as white petrolatum or mineral oil, or (2) an absorbent base, i.e., one consisting of an anhydrous substance or substances which can absorb water, for example anhydrous lanolin. Customarily, following formation of the base, whether oleaginous or absorbent, the active ingredient or ingredients (compound or compounds) is added to an amount affording the desired concentration.

Creams are oil/water emulsions. They consist of an oil phase (internal phase), comprising typically fixed oils, hydrocarbons, and the like, such as waxes, petrolatum, mineral oil, and the like, and an aqueous phase (continuous phase), comprising water and any water-soluble substances, such as added salts. The two phases are stabilized by use of an emulsifying agent, for example, a surface active agent, such as sodium lauryl sulfate; hydrophilic colloids, such as acacia colloidal clays, veegum, and the like. Upon formation of the emulsion, the active ingredient or ingredients (compound or compounds) customarily is added to an amount to achieve the desired concentration.

Gels comprise a base selection from an oleaginous base, water, or an emulsion-suspension base. To the base is added a gelling agent which forms a matrix in the base, increasing its viscosity. Examples of gelling agents are hydroxypropyl cellulose, acrylic acid polymers, and the like. Customarily, the active ingredient or ingredients (compound or compounds) is added to the formulation at the desired concentration at a point preceding addition of the gelling agent.

The amount of compound or compounds incorporated into a topical formulation is not critical; the concentration should only be a range sufficient to permit ready application of the formulation to the affected tissue area in an amount which will deliver the desired amount of compound or compounds.

The customary amount of a topical formulation to be applied to an affected tissue will depend upon an affected tissue size and concentration of compound or compounds in the formulation. Generally, the formulation will be applied to the effected tissue in an amount affording from about 1 to about 500 µg compound or compounds per cm^2 of an affected tissue. Preferably, the applied amount of compound will range from about 30 to about 300 $\mu g/cm^2$, more preferably, from about 50 to about 200 $\mu g/cm^2$, and, most preferably, from about 60 to about 100 $\mu g/cm^2$.

The following formulation examples are illustrative only and are not intended to limit the scope of the invention in any way.

Formulation 1

Hard gelatin capsules are prepared using the following ingredients:

	Quantity (mg/capsule)
Active agent or agents	250
starch, dried	200
magnesium stearate	10
Total	460 mg

The above ingredients are mixed and filled into hard gelatin capsules in 460 mg quantities.

Formulation 2

A tablet is prepared using the ingredients below:

	Quantity (mg/capsule)
Active agent or agents	250
cellulose, microcrystalline	400
silicon dioxide, fumed	10
stearic acid	5
Total	665 mg

The components are blended and compressed to form tablets each weighing 665 mg.

Formulation 3

An aerosol solution is prepared containing the following components:

	Quantity (mg/capsule)
Active agent or agents	0.25
Ethanol	29.75
Propellant 22 (chlorodifluoromethane)	70.00
Total	100.00

The active compound is mixed with ethanol. The mixture is added to a portion of the Propellant 22, cooled to -30°C and transferred to a filling device. The required amount is then fed to a stainless steel container and diluted with the remainder of the propellant. The valve units are then fitted to the container.

Formulation 4

Tablets each containing 60 mg of active ingredient are made as follows:

	Quantity (mg/capsule)
Active agent or agents	60 mg
starch	45 mg
microcrystalline cellulose	35 mg
polyvinylpyrrolidone (as 10% solution in water)	4 mg
sodium carboxymethyl starch	4.5 mg
magnesium stearate	0.5 mg
talc	1 mg
Total	150 mg

The active ingredient or ingredients, starch and cellulose are passed through a No. 45 mesh U.S. sieve and mixed thoroughly. The solution of polyvinylpyrrolidone is mixed with the resultant powders which are then passed through a No. 14 mesh U.S. sieve. The granules so produced are dried at 50°C and passed through a No. 18 mesh U.S. sieve. The sodium carboxymethyl starch, magnesium stearate and talc, previously passed through a No. 60 mesh U.S. sieve, are then added to the granules which, after mixing, are compressed on a tablet machine to yield tablets each weighing 150 mg.

Formulation 5

Capsules each containing 80 mg of medicament are made as follows:

	Quantity (mg/capsule)
Active agent or agents	80 mg
Starch	59 mg
microcrystalline cellulose	59 mg
magnesium stearate	2 mg
Total	200 mg

The active ingredient or ingredients, cellulose, starch and magnesium stearate are blended, passed through a No. 45 mesh U.S. sieve, and filled into hard gelatin capsules in 200 mg quantities.

Formulation 6

Suppositories each containing 225 mg of active ingredient or ingredients may be made as follows:

	Quantity (mg/capsule)
Active agent or agents	225 mg
saturated fatty acid glycerides	2,000 mg
Total	2,225 mg

The active ingredient or ingredients are passed through a No. 60 mesh U.S. sieve and suspended in the saturated fatty acid glycerides previously melted using the minimum heat necessary. The mixture is then poured into a suppository mold of nominal 2 g capacity and allowed to cool.

Formulation 7

Suspensions each containing 50 mg of medicament per 5 mL dose are made as follows:

	Quantity (mg/capsule)
Active agent or agents	50 mg
sodium carboxymethyl cellulose	50 mg
syrup	1.25 mL
benzoic acid solution	0.10 mL
flavor	q.v.
color	q.v.
purified water to total	5 mL

The medicament or medicaments are passed through a No. 45 mesh U.S. sieve and mixed with the sodium carboxymethyl cellulose and syrup to form a smooth paste. The benzoic acid solution, flavor and color are diluted with some of the water and added, with stirring. Sufficient water is then added to produce the required volume.

Formulation 8

An intravenous formulation may be prepared as follows:

	Quantity (mg/capsule)
Active agent or agents	250 mg
isotonic saline	1000 mg

The solution of the above ingredients is administered intravenously at a rate of 1 mL per minute to a subject in need of treatment.

We claim:

1. A pharmaceutical composition comprising an inhibitor of protein kinase C or a pharmaceutically acceptable salt thereof, and an antioxidant, essential fatty acid, or prostacyclin agent, or a pharmaceutically acceptable salt thereof, in combination with a pharmaceutically acceptable carrier, diluent, or excipient.

2. A pharmaceutical composition according to claim 1, wherein the inhibitor of protein kinase C is selective for the β-isozyme of protein kinase C.

3. A method of inhibiting protein kinase C, comprising administering to a mammal in need of such treatment a pharmaceutically effective amount of an inhibitor of protein kinase C, or a pharmaceutically acceptable salt thereof, and a pharmaceutically effective amount of an antioxidant, essential fatty acid, or prostacyclin agent, or a pharmaceutically acceptable salt thereof.

4. A method of inhibiting protein kinase C, comprising administering to a mammal in need of such treatment a pharmaceutically effective amount of an inhibitor of protein kinase C, or a pharmaceutically acceptable salt thereof, and a pharmaceutically effective amount of an antioxidant, or a pharmaceutically acceptable salt thereof.

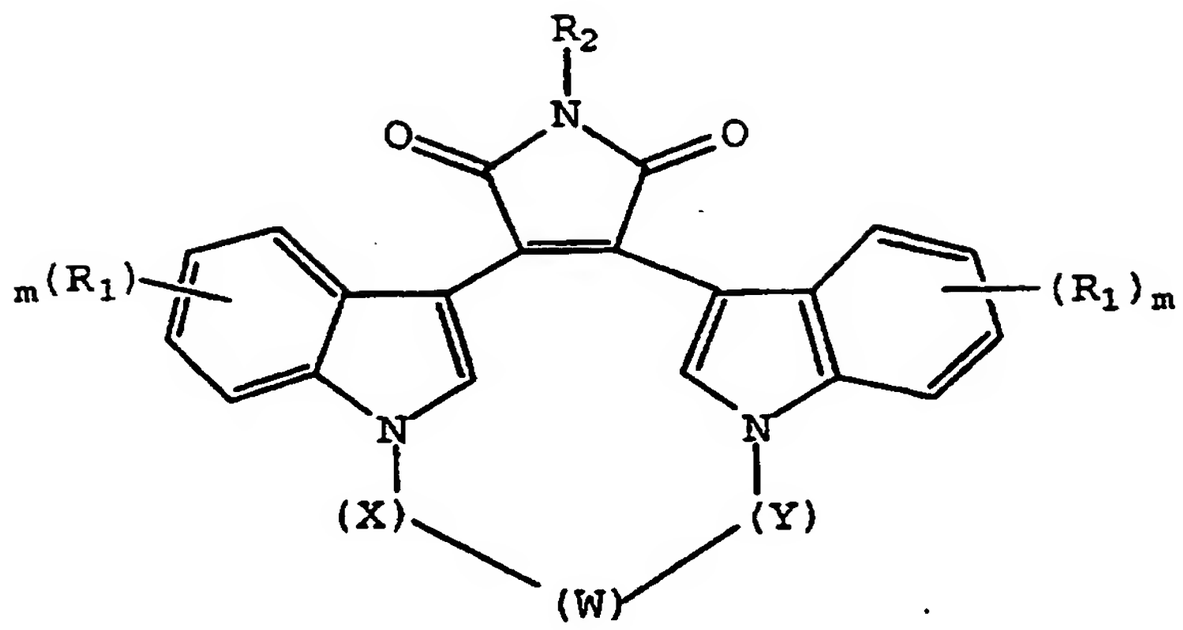
5. A method of inhibiting protein kinase C, comprising administering to a mammal in need of such treatment a pharmaceutically effective amount of an inhibitor of protein kinase C, or a pharmaceutically acceptable salt thereof, and a pharmaceutically effective amount of an essential fatty acid, or a pharmaceutically acceptable salt thereof.

6. A method of inhibiting protein kinase C, comprising administering to a mammal in need of such treatment a pharmaceutically effective amount of an inhibitor of protein kinase C, or a pharmaceutically acceptable salt thereof, and a pharmaceutically effective amount of a prostacyclin agent, or a pharmaceutically acceptable salt thereof.

7. A method for treating a condition associated with diabetes mellitus or its complications comprising administering to a mammal in need of such treatment a pharmaceutical composition according to claim 1.

8. A method for treating a condition associated with diabetes mellitus or its complications comprising administering to a mammal in need of such treatment a pharmaceutically effective amount of an inhibitor of protein kinase C, or a pharmaceutically acceptable salt thereof, and a pharmaceutically effective amount of an antioxidant, essential fatty acid, or prostacyclin agent, or a pharmaceutically acceptable salt thereof.

9. A composition according to claim 1 or 2, or a method according to claim 3, 4, 5, 6, 7, 8 or 14 wherein the inhibitor of protein kinase C is a compound of the formula



(I)

wherein:

W is -O-, -S-, -SO-, -SO_2-, -CO-, C_2-C_6 alkylene, substituted alkylene, C_2-C_6 alkenylene, -aryl-, -aryl$(CH_2)_m$O-, -heterocycle-, -heterocycle-$(CH_2)_m$O-, -fused bicyclic-, -fused bicyclic-$(CH_2)_m$O-, -NR_3-, -NOR_3-, -CONH-, or -NHCO-;

X and Y are independently C_1-C_4 alkylene, substituted alkylene, or together X, Y, and W combine to form -$(CH_2)_n$-AA-;

R_1 is independently hydrogen, halo, C_1-C_4 alkyl, hydroxy, C_1-C_4 alkoxy, haloalkyl, nitro, NR_4R_5, or -NHCO(C_1-C_4 alkyl);

R_2 is hydrogen, CH_3CO-, NH_2, or hydroxy;

R_3 is hydrogen, $(CH_2)_m$aryl, C_1-C_4 alkyl, -COO(C_1-C_4 alkyl), -$CONR_4R_5$, -(C=NH)NH_2, -SO(C_1-C_4 alkyl), -$SO_2(NR_4R_5)$, or -SO_2(C_1-C_4 alkyl);

R_4 and R_5 are independently hydrogen, C_1-C_4 alkyl, phenyl, benzyl, or combine to the nitrogen to which they are bonded to form a saturated or unsaturated 5 or 6 member ring;

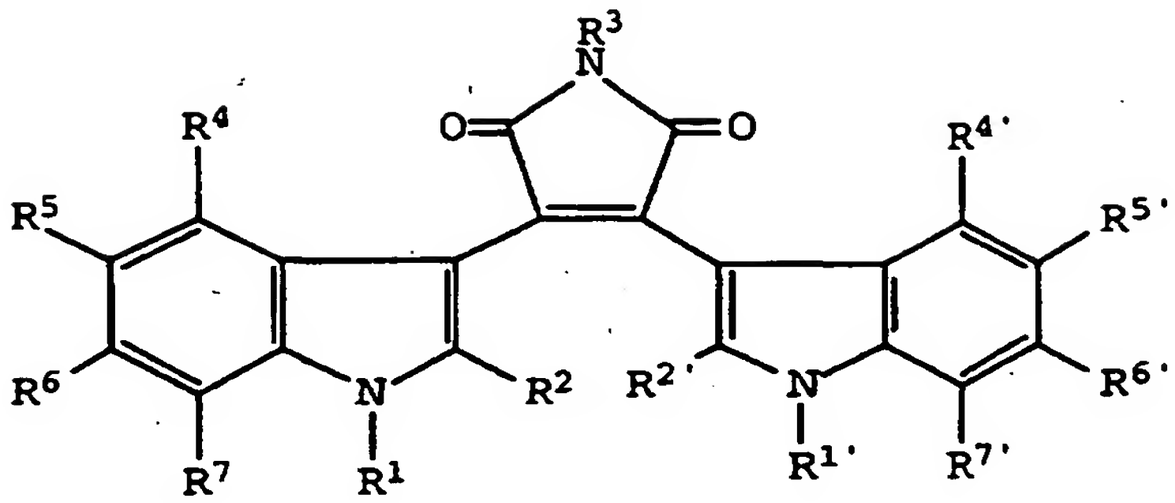
AA is an amino acid residue;

m is independently 0, 1, 2, or 3; and

n is independently 2, 3, 4, or 5,

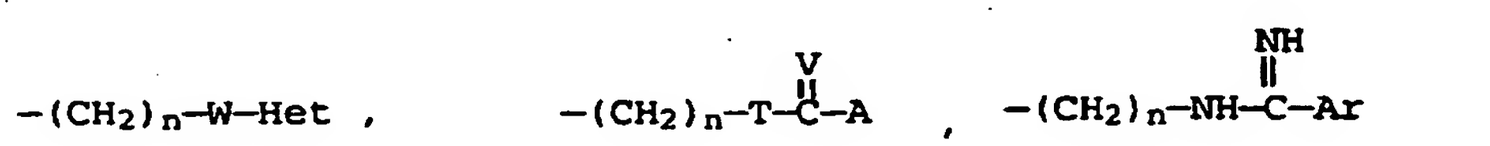
or a pharmaceutically acceptable salt thereof;

or a compound of the formula



wherein:

R^1 and $R^{1'}$ are independently hydrogen, alkyl, haloalkyl, alkenyl, arylalkyl, alkoxyalkyl, hydroxyalkyl, aminoalkyl, monoalkylaminoalkyl, dialkylaminoalkyl, acylaminoalkyl, acyloxyalkyl, cyanoalkyl, amidinoalkyl, carboxyalkyl, alkoxycarbonylalkyl, aminocarbonylalkyl, or a group of the formula:



(a) (b) (c)

Het signifies a heterocyclyl group;

W signifies NH, S or a bond;

T signifies NH or S;

V signifies O, S, NH, or NCN;

A signifies alkylthio, amino, monoalkylamino or dialkylamino;

Ar signifies aryl;

R^2 and $R^{2'}$ are independently hydrogen, alkyl, alkoxyalkyl, hydroxyalkyl, C_1-C_3 alkylthio, $S(0)C_1$-C_3 alkyl, CF_3; or R^1 and R^2 can combine to form $-(CH_2)_r-X-CH_2-$;

R^3 is hydrogen or CH_3CO;

R^4, $R^{4'}$, R^5, $R^{5'}$, R^6, $R^{6'}$, R^7 and $R^{7'}$ are independently hydrogen, halogen, alkyl, hydroxy, alkoxy, $-COO(C_1$-C_3 alkyl), CF_3, nitro, amino, acetylamino, monoalkylamino, dialkylamino, alkylthio, C_1-C_3 alkylthio, or $S(0)C_1$-C_3 alkyl;

X is CHR^8 or NR^8;

R^8 is $(CH_2)_sR^9$;

R^9 is hydrogen, hydroxy, alkoxy, amino, monoalkylamino, dialkylamino, trialkylamino, azido, acylamino, alkoxycarbonyl, cyano, amidino, or aminocarbonyl;

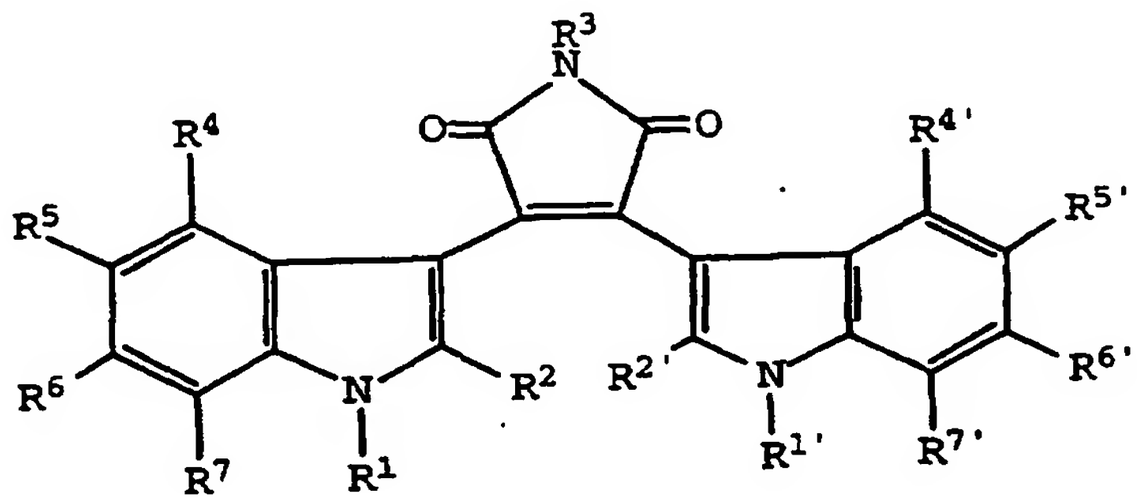
n is 1, 2, 3, 4, 5 or 6;

r is 1, 2, or 3; and

s is 0, 1, 2 or 3,

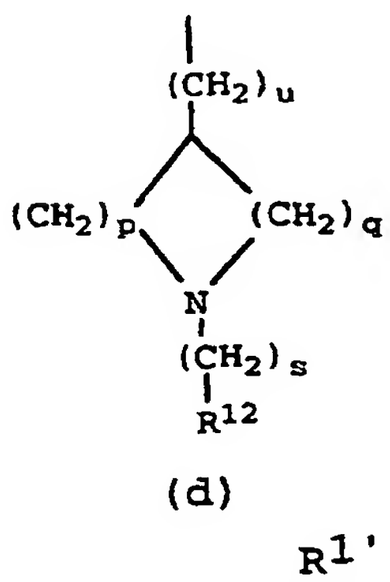
or a pharmaceutically acceptable salt thereof;

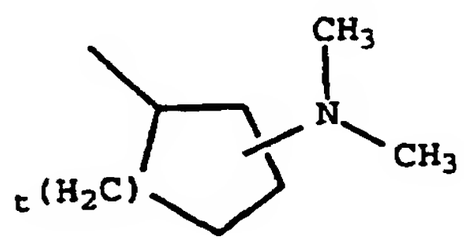
or a compound of the formula

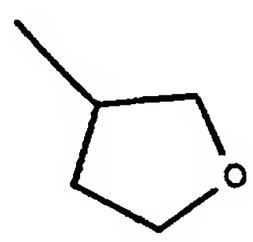


wherein:

R^1 is







(d) (e) or (f);

$R^{1'}$ is hydrogen, C_1-C_4 alkyl, aminoalkyl, monoalkylaminoalkyl, or dialkylaminoalkyl;

R^2 and $R^{2'}$ are independently hydrogen, alkyl, alkoxyalkyl, hydroxyalkyl, C_1-C_3 alkylthio, $S(0)C_1$-C_3 alkyl, CF_3;

R^3 is hydrogen or CH_3CO-;

R^4, $R^{4'}$, R^5, $R^{5'}$, R^6, $R^{6'}$, R^7 and $R^{7'}$ are independently hydrogen, halogen, alkyl, hydroxy, alkoxy, -COO(C_1-C_3 alkyl), CF_3, nitro, amino, acetylamino, monoalkylamino, dialkylamino, alkylthio, C_1-C_3 alkylthio, or S(0)C_1-C_3 alkyl;

R^{12} is hydrogen, alkyl, haloalkyl, cycloalkyl, acetyl, aryl, -CH(aryl)$_2$, amino, monoalkylamino, dialkylamino, guanidino, -C(=N(alkoxycarbonyl))NH(alkyoxycarbonyl), amidino, hydroxy, carboxy, alkoxycarbonyl or heterocyclyl;

p and q are independently 1, 2, 3, or 4;

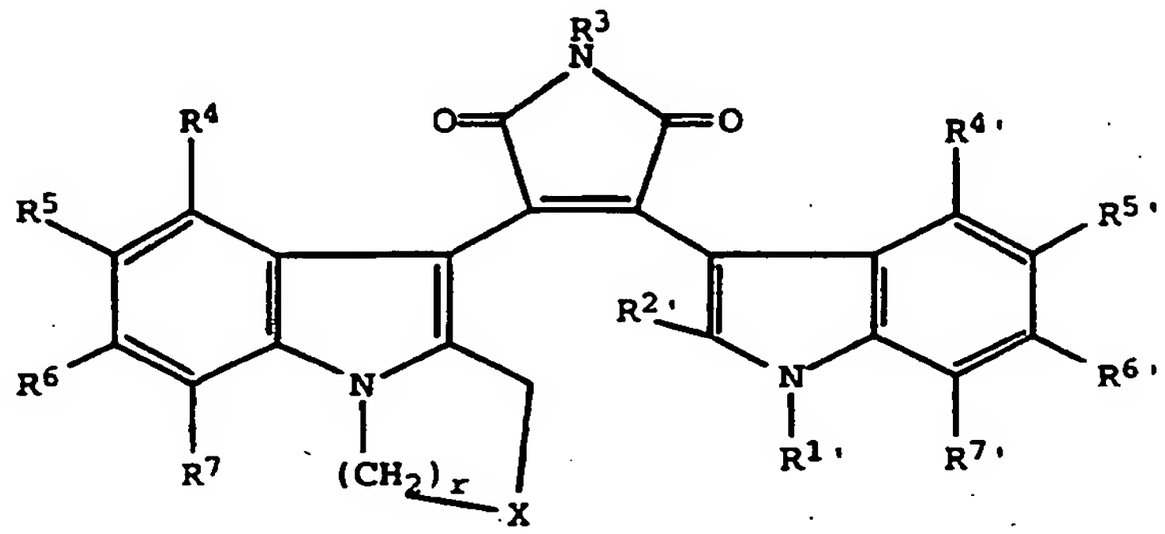
s is 0, 1, 2 or 3;

t is 1 or 2;

u is 0 or 1; or

a pharmaceutically acceptable salt thereof;

or a compound of the formula



wherein:

$R^{1'}$ is hydrogen, C_1-C_4 alkyl, aminoalkyl, monoalkylaminoalkyl, or dialkylaminoalkyl;

$R^{2'}$ is hydrogen, alkyl, alkoxyalkyl, hydroxyalkyl, C_1-C_3 alkylthio, S(0)C_1-C_3 alkyl, CF_3;

R^3 is hydrogen or CH_3CO-;

R^4, $R^{4'}$, R^5, $R^{5'}$, R^6, $R^{6'}$, R^7 and $R^{7'}$ are independently hydrogen, halogen, alkyl, hydroxy, alkoxy, -COO(C_1-C_3 alkyl), CF_3, nitro, amino, acetylamino, monoalkylamino, dialkylamino, alkylthio, C_1-C_3 alkylthio, or S(0)C_1-C_3 alkyl;

X is CR^8R^9;

R^8 is $(CH_2)_sR^{10}$;

R^9 is $(CH_2)_sR^{11}$;

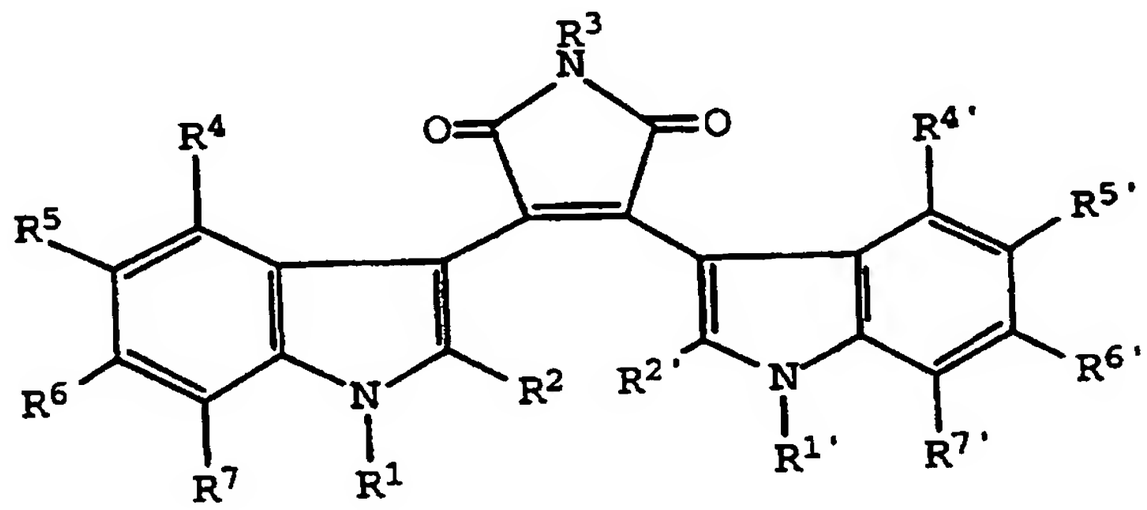
R^{10} and R^{11} are independently hydroxy, alkoxy, carboxy, acyloxy, amino, monoalkylamino, dialkylamino, trialkylamino, azido, acylamino, alkoxycarbonyl, cyano, amidino, or aminocarbonyl;

r is 1, 2, or 3;

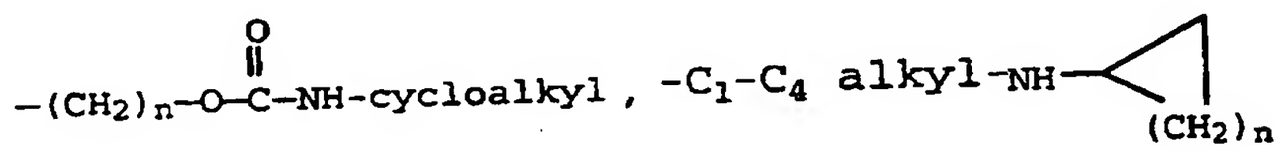
s is 0, 1, 2 or 3; or

a pharmaceutically acceptable salt thereof;

or a compound of the formula



wherein:

R^1 is  or alkylglycose residue;

$R^{1'}$ is hydrogen, C_1-C_4 alkyl, cyclopropylmethyl, aminoalkyl, monoalkylaminoalkyl, or dialkylaminoalkyl;

R^2 and $R^{2'}$ are independently hydrogen, alkyl, alkoxyalkyl, hydroxyalkyl, C_1-C_3 alkylthio, $S(0)C_1$-C_3 alkyl, CF_3;

R^3 is hydrogen or CH_3CO-;

R^4, $R^{4'}$, R^5, $R^{5'}$, R^6, $R^{6'}$, R^7 and $R^{7'}$ are independently hydrogen, halogen, alkyl, hydroxy, alkoxy, -$COO(C_1$-C_3 alkyl), CF_3, nitro, amino, acetylamino, monoalkylamino, dialkylamino, alkylthio, C_1-C_3 alkylthio, or $S(0)C_1$-C_3 alkyl;

n is 1, 2, 3, 4, 5 or 6; or
a pharmaceutically acceptable salt thereof.

10. A composition according to claim 1 or 2, or a method according to claim 3, 4, 5, 6, 7 or 8, wherein the inhibitor of protein kinase C is

(S)-3,4-[(N,N'-1,1'-((2''-ethoxy)-3'''(O)-4'''(N,N-dimethylamino)-butane)-bis-(3,3'-indolyl)]-1(H)pyrrole-2,5-dione,

3-[1-(1-benzyl-piperidin-4-yl)indol-3-yl]-4-(1-methyl-indol-3-yl)-pyyrole-2,5,dione,

3-[1-(1-cyclopropylmethylene-piperidin-4-yl)indol-3-yl]-4-(1-methyl-indol-3-yl)-pyyrole-2,5,dione,

3-[1-(1-methyl-piperidin-4-yl)indol-3-yl]-4-(1-methyl-indol-3-yl)-pyyrole-2,5,dione,or

3-[1-(1-(pyridin-2-ylmethyl)piperidin-4-yl)indol-3-yl]-4-(1-methyl-indol-3-yl)-pyyrole-2,5,dione, or

a pharmaceutically acceptable salt thereof.

11. A composition according to claim 1, 2, 9 or 10, or a method according to claim 3, 4, 5, 6, 7 , 8 or 14 wherein the antioxidant is vitamin E, probucol, butylated hydroxytoluene, beta-carotene, lycopene, silybin, diosmin, hesperidin, delphinidin), ubiquinol, tirilazad, a 21-aminosteroid, lipoic acid, vitamin C, superoxide dismutase, dihydrolipoic acid, acetyl-L-cysteine, glutathione,dimethylthiourea, deferoxamine, trientine, or aminoguanidine or a derivative thereof.

12. A composition according to claim 1, 2, 9 or 10, or a method according to claim 3, 4, 5, 6, 7, 8, or 14 wherein the essential fatty acid is γ-linolenic acid or arachidonic acid.

13. A composition according to claim 1, 2, 9 or 10, or a method according to claim 3, 4, 5, 6, 7 , 8 or 14 wherein the prostacyclin agent is a type III phosphodiesterase inhibitor, iloprost or beraprost sodium.

14. A method according to claim 7 or 8 wherein the condition is diabetic neuropathy, vascular complications, erectile dysfunction, ischemia, inflammation, central nervous system disorders, cardiovascular disease, Alzheimer's Disease, dermatological disease or cancer.

15. The use of a composition according to claim 1, 2, 9, 10, 11, 12 or 13 in the preparation of a medicament for treating conditions associated with diabetes mellitus or its complications.

16. A composition according to claim 1, 2, 9, 10, 11, 12 or 13, or a method according to claim 3, 4, 5, 6, 7, 8 or 14 wherein the ratio of the amount of the inhibitor of protein kinase C to the amount of antioxidant, essential fatty acid or prostacyclin agent is from 100 to 1, to 1 to 100.

17. A composition according to claim 1, 2, 9, 10, 11, 12 or 13, or a method according to claim 3, 4, 5, 6, 7, 8 or 14 wherein the ratio of the amount of the inhibitor of protein kinase C to the amount of antioxidant, essential fatty acid or prostacyclin agent is from 10 to 1, to 1 to 10.

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(54) Title: THERAPEUTIC COMPOSITIONS INCLUDING PROTEIN KINASE C INHIBITORS

(57) Abstract: Compositions comprising a PKC inhibitor, or a pharmaceutically acceptable salt thereof, and an antioxidant, essential fatty acid, or a prostacyclin agent, or a pharmaceutically acceptable salt thereof are provided. Also provided are methods of treatment comprising administration of such compositions, and methods of treatment comprising co-administration of a PKC inhibitor, or a pharmaceutically acceptable salt thereof, and an antioxidant, essential fatty acid, or a prostacyclin agent, or a pharmaceutically acceptable salt thereof.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 00/26254

A. CLASSIFICATION OF SUBJECT MATTER
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According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, PAJ, WPI Data, BIOSIS, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
A	S.E.BURSELL, G.L.KING: "Can protein kinase C inhibition " DIABETES RESEARCH CLINICAL PRACTICE, vol. 45, no. 2-3, 1999, pages 169-182, XP001010001 page 169 page 174 page 176 page 177, column 1 --- -/--	1

[X] Further documents are listed in the continuation of box C

[ ] Patent family members are listed in annex

* Special categories of cited documents

"A" document defining the general state of the art which is not considered to be of particular relevance

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
29 June 2001	20/07/2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx 31 651 epo nl, Fax: (+31-70) 340-3016	Peeters, J

2

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 00/26254

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category ~	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
A	K.HEHENBERGER, A.HANSSON: "High glucose-induced growth factor resistance in human fibroblasts can be reversed by antioxidants and protein kinase C-inhibitors" CELL BIOCHEMISTRY AND FUNCTION, vol. 15, no. 3, 1997, pages 197-201, XP001010004 page 197 page 198, column 2 page 199, column 2 page 200, column 1 -----	1